

# **CHARACTERIZATION OF MECHANISMS INFLUENCING CANNIBALISM AMONG LARVAL AMPHIBIANS**

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfilment of  
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By

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## Abstract

Cannibalism is a seemingly aberrant interaction, appearing counter to the fitness of individuals. Yet cannibalism is not overly uncommon, and naturally occurs among aquatic organisms, including larval amphibians. In temporary wetlands larval amphibians are in a race to complete metamorphosis before their aquatic habitat disappears. When intraspecific competition intensifies, eating conspecifics may represent a beneficial if not necessary strategy. The research presented within this thesis aims to characterize factors that influence cannibalism within populations of larval amphibians. Wood frog tadpoles (*Lithobates sylvaticus*) were used to test potential benefits of cannibalism as a diet, determine if dietary quality and nutritional stress influence cannibalism, and investigate the roles of competition and chemical cues in influencing cannibalism. Larval long-toed salamanders (*Ambystoma macrodactylum*), and ringed salamanders (*A. annulatum*) were used to investigate a functional link between trophic polymorphism and cannibalism in natural populations. Results suggest that perceived increases in competition may stimulate some individuals to become less risk averse, and more aggressive, which may in turn facilitate cannibalistic behaviour. Cannibalism itself provided only conditional benefits to larval wood frogs, rather than the optimal growth that would be expected from an ideal diet. However, this may have been the result of individual variation in response to the diet and/or conspecific cues as opposed to a nutritional deficit. In conditions where tadpoles could perceive increased competition they altered their behaviour and morphology in ways that may improve their foraging success and potentially promote cannibalism. Finally, a functional link appears to exist between head morphology and cannibalism in natural wetlands. However, the appearance of this morphology appears related to conditions that may facilitate increased population densities through rapid pond drying.

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# **Chapter 1: Introduction**

## **1.1 General Introduction**

Animals experience risk on a continuous basis; they may be unsuccessful in their acquisition of resources, or fail in an opportunity to reproduce, and this may negatively impact their fitness in a relatively minor capacity over the long-term (Dawkins and Krebs, 1979; Lima and Dill, 1989). However, some failures are completely unforgiving. The inability to avoid lethal risks will have a far more negative and lasting impact upon the animals' fitness (Lima and Dill, 1989). Thus, predation imposes a disproportionately negative impact upon prey species, which may lose their lives while predatory species may simply miss a meal (the life-meal principle) (Dawkins and Krebs, 1979; Lima and Dill, 1989; Ferrari et al., 2010). The result may be an “arms race” between predators and prey, whereby each side successively exhibits adaptations to detect one another and either promote or deter predatory success, respectively (Lima and Dill, 1989; Ferrari et al., 2010). Intraspecific competition too presents a strong selective force, favouring those with adaptations that provide a competitive advantage in the acquisition of specific resources, and provoking adaptations among individuals within a population (Dawkins and Krebs, 1979).

Habitat condition can intensify interactions among organisms. Temporary wetlands are inherently transient, and through seasonal drying may impose limitations to spatial and dietary resources available to occupant species (Enriquez-Urzelai et al., 2013; Griffiths, 1997; Wellborn et al., 1996). Species occupying temporary wetlands require adaptations, or life histories that allow for their survival of drying periods, those that do not are naturally excluded (Griffiths, 1997; Wellborn et al., 1996). Beyond this, species occupying temporary wetlands must be capable of interpreting and evaluating risk, and then responding rapidly and efficiently (Relyea, 2004; Richter-Boix et al., 2007). As temporary wetlands gradually

disappear, resource abundance may decrease while population density increases (Wildy, 2001). Many species of amphibians frequently utilize temporary wetlands for larval development. Under such deteriorating conditions, acceleration of development to metamorphosis is paramount to escape the drying habitat; however, limitations to food may restrict their ability to do so (Enriquez-Urzelai et al., 2013). Under such conditions, individuals with adaptations allowing them to successfully acquire food may survive to metamorphosis. Intraspecific predation may then represent an extreme form of interference competition (Fox, 1975a). Conspecific tissues may also sustain individuals to survive conditions of dietary stress, and provide benefits to growth and/or development (Bleakney, 1958; Meffe and Crump, 1987).

## **1.2 Intraspecific Predation**

Predation has traditionally been viewed as a linear interaction where energy and nutrients are directed upwards through trophic levels, from producers to apical predators (Pimm, 1982; Polis, 1991). Intraspecific predation (cannibalism) may be considered “a short-circuited predator-prey system”, also known as a self-loop (Claessen et al., 2003; Polis, 1991). Trophic looping is a pathway of feeding interactions involving one or more species where a species represents both predator and prey (e.g. cannibalism, mutual predation between species, etc.) (Polis, 1991). Such trophic structures were previously dismissed by many ecologists as “unreasonable structures” (Polis, 1991). For example, Pimm (1982) stated “I know of no cases, in the real world, with loops”; though he later revised this view to concede the occurrence of loops in age structured aquatic systems, but maintained loops were rare in terrestrial systems (Pimm and Rice, 1987). However, Polis (1991) notes that cannibalism has been reported in over 1300 species, and is potentially vital to population dynamics of many communities. Cannibalism has been reported among a wide diversity of



organisms including herpetofauna, fishes, birds, invertebrates, and mammals (Crump, 1986; Fox, 1975a; Polis, 1981; Polis and Myers, 1985; Polis et al., 1984).

Cannibalism, typically involves larger, healthier individuals consuming smaller and/or weakened individuals (Claessen et al., 2003; Polis, 1981). However, instances of larger individuals attacked by swarms of smaller conspecifics have been observed (Polis, 1981). Crump (1986) observed younger tadpoles opportunistically attacking and consuming tails and developing limbs of vulnerable metamorphosing tadpoles, and initial attacks invoked recruitment of additional tadpoles to further cannibalize victims. Such instances of group cannibalism have been described in numerous species of insects, several tadpole species, fishes, and in primates (Cuff, 1980; Duelli, 1981; Goodall, 1977; Polis, 1981; Pomeroy, 1981; Sikand and Ranade, 1975). Some species may also exhibit incomplete predation whereby agonistic behaviour escalates to ingestion of conspecific tissues (e.g., tail biting in larval amphibians, and fin and/or scale biting in fish) (Polis, 1981). Oophagy, the consumption of developing or non-viable eggs is commonly observed in amphibians, sharks, boney fishes, and invertebrates (Bass et al., 1975; Bonnet et al., 2008; Balon, 1981; Crump, 1983; Dulvy and Reynolds, 1997; Gilmore, 1983; Gilmore, 1993; Gibson, 1997; Marty et al., 1997; Szeinfeld, 1993; Strathmann and Strathmann, 2006). Cannibalistic necrophagy is also relatively common, and involves scavenging tissues from dead conspecifics (Lockwood, 1989). Larval amphibians and fish species are commonly observed consuming dead or dying conspecifics (Brunner et al., 2007; Crump, 1983; Crump, 1986; Diamant, 1997). Many invertebrates exhibit necrophagy, and may actively seek out cadavers (necrophily) as a food source (Brown and Norris, 2004; Lockwood, 1989).

Generalized conditions have been suggested that potentially encourage or invoke cannibalistic behaviour (Fox, 1975a; Møller et al., 2008). These conditions include reductions in food availability, increased density/crowding, conspicuous victim behaviour, stress, and

increased victim availability (Crump, 1983; Fox, 1975a, b; Møller et al., 2008). Scarcity of food is a prominent factor influencing the expression of cannibalism; however, Fox (1975a) indicated that starvation was not necessary for its initiation. In many species, the frequency of cannibalism is inversely related to density of alternative prey, and cannibalistic behaviour commences to reduce population size before starvation occurs (Armstrong, 1964; Bulkley, 1970; Fox, 1975b). Additionally, organisms occupying temporary habitats (i.e., ephemeral wetlands) are believed to become cannibals in order to improve growth and development, and thus enhance survival (Church and Sherratt, 1996; Crump, 1983; Crump, 1990; Michimae and Wakahara, 2001; Wildy et al. 2001). This is supported by Richter-Boix (2007) who found that tadpoles selectively chose diets, which either promoted increased growth or enhance development in response to specific environmental and ecological pressures.

Crowding effects can be confounded with those of food shortage (Fox, 1975a). Multiple studies indicate that crowding can influence the frequency of cannibalism independent of the presence of alternate prey (Fox, 1975a; Michimae and Wakahara, 2001; Wildy et al., 2001). This may be due to spatial limitations, increased encounters with conspecific prey, and/or increased cues (i.e. tactile or chemical) that promote cannibalism (Fox, 1975a; Hoffman and Pfennig, 1999; Thibault, 1974). However, Kipling and Frost (1970) observed that the amount of alternative prey did reduce cannibalism rates of pike.

Availability and behaviour of victims may be related to their age, size, condition, and relationship to cannibals (Fox, 1975a; Keren-Rotem et al., 2006; Michimae and Wakahara, 2001). Fox (1975a) indicates that non-cannibals who lack an antipredator response when cannibals are present will increase the occurrence of cannibalism. Naïve individuals that have not been conditioned to cannibals, newly hatched individuals, and the sick or injured may not attempt to evade cannibalistic individuals and may be at increased risk of predation (Fox, 1975a; Keren-Rotem et al., 2006). Keren-Rotem et al. (2006) noted that young chameleons

typically avoided habitats occupied by larger adults until they had matured, and small juveniles entering adult territory were at risk of cannibalism by adults. Similarly, individuals may cannibalize conspecifics if they are available, irrespective of other factors (Brower, 1961; Keren-Rotem et al., 2006). Brower (1961) observed that newly hatched butterfly larvae would consume unhatched conspecifics within their immediate feeding range even at low population densities, and while consuming their typical plant diet.

Fox (1975a) indicates that association of physiological and psychological stress has been described in a few field situations. Boice (1972) observed that social ranking of Norway rats influenced cannibalism; lower ranking females cannibalized the majority (60%) of their offspring, while higher ranking females raised all of their offspring. Clark (1967) found that physiologically stressed grasshoppers in Australia consumed both living and dead conspecifics, while individuals that showed no signs of stress did not cannibalize.

### **1.2.1 Potential Benefits**

There are a number of generalized benefits to cannibalistic populations under appropriate circumstances. These benefits include enhancement of growth and development, reduced interaction intensity among individuals, increased survivorship, establishment of social dominance, and regulation of population dynamics (Fox, 1975a, b).

Consuming conspecific tissues can offer an abundant, all-in-one diet, providing the “materials necessary for growth, maintenance, and reproduction” (Meffe and Crump, 1987; Michimae and Wakahara, 2001). Conspecific tissues should therefore minimize the diversity of prey required to provide all the necessary nutrients, including all essential and most, if not all non-essential amino acids thereby maximizing the efficiency of growth and/or development (Church and Sherratt, 1996; Giese and Alden, 1938; Pfennig et al., 1991; Smith and Skúlason, 1996). Alternatively, increased growth achieved through cannibalism may

reduce predation pressure, outgrowing predators, and may broaden the diversity of prey these individuals are capable of consuming (Crump, 1990; Parris et al., 2005; Walls et al., 1993; Wildy et al., 1998). Additionally, intraspecific competition intensity can be reduced as a result of cannibalism within the population (Fox, 1975a; Wildy et al., 2001). Cannibalism reduces population density (through intraspecific predation), provides novel prey to cannibalistic individuals (conspecifics), and may reduce utilization of optimal resources as a result of the aforementioned effects as well as antipredator behaviours by non-cannibals (Wildy et al., 1999). Antipredator behaviour observed in larval salamanders appears to impose negative impacts on non-cannibals; Wildy et al. (1999) observed slower growth rates and longer times to metamorphosis.

Rapid development of individuals within highly fluctuating habitats can improve survivorship by allowing for early maturation and habitat escape (Crump, 1983). In temporary wetlands, cannibalistic tadpoles may reach metamorphosis earlier, reducing risk of mortality from desiccation (Crump, 1983). Church and Sherratt (1996) observed significantly increased longevity in cannibalistic mosquito larvae, suggesting cannibalism potentially improves larval survivorship to maturation. Similarly, Meffe and Crump (1987) observed increased the growth and improved reproductive success in cannibalistic mosquito fish.

Cannibalism may also control and structure populations (Polis, 1981; Trumbo and Fernandez, 1995; Vitt, 2000). Cannibalism may facilitate individual survival under conditions of high competition and/or low food availability (Fox, 1975a). Observations of sheep blowfly populations in the laboratory indicated cannibalistic species were able persist under unfavorable conditions (food scarcity), whereas non-cannibalistic species produced smaller individuals and were unable to maintain populations under conditions of high competitive intensity (Fox, 1975a). Fox (1975a) asserts that cannibalism is both an intraspecific predator-

prey interaction, but also a form of interference competition, “limiting population size before the resource itself becomes limiting.”

### **1.2.2 Possible Costs and Consequences**

Cannibalism carries inherent consequences that may limit its expression within populations. Cannibalism is an aggressive behaviour, which is inherently associated with the risk of reciprocated or defensive aggression from the victim (McAdie and Keeling, 2000). Although there is often a potential risk of injury to a predator from interspecific predator-prey interactions, attacking a conspecific with similar characteristics may present an increased risk (Pfennig et al., 1998; Wildy et al., 1998).

Cannibalistic individuals have been suggested to potentially suffer reduced fitness by consuming closely related kin (Fox, 1975a; Wildy et al., 1998). Fox (1975a) indicated that if cannibals express aggression that results in the elimination of their kin (siblings and/or offspring), or do so with greater frequency than unrelated individuals, this will reduce their fitness through reduction in abundance, or persistence of their genotype. However, cannibalistic species may also exhibit some degree of kin selection, reducing predation on siblings and other close relations (Pfennig and Collins, 1994).

Cannibalism of sick, dying, or dead individuals provides an opportunistic source of nutrients; however it also presents a relatively high risk of pathogen transmission among individuals and has been suggested as a potential limiting factor or selective force against cannibalism (Meffe and Crump, 1987; Fox, 1975a, b; 1987; Parris et al., 2005; Pfennig et al., 1991; Pfennig et al., 1998). Cannibalism-mediated pathogen transmission is a serious health concern to humans (kuru) and many animals of socio-economic value (i.e. cattle; bovine spongiform encephalopathy) (Holt and Phillips, 1988). Many pathogens exhibit some degree

of host specificity, and transmission can be enhanced by ingestion, as opposed to contact (Bolker et al., 2008; Williams and Hernández, 2006).

### **1.2.3 Selection and Evolution of Cannibalism**

In many species, there appears to be an underlying genetic basis that influences the expression of cannibalism (Polis, 1981). This is evidenced by differences in “cannibalistic tendencies” observed in strains or breeds of rotifers, arthropods [flour beetles (*Tribolium*), and mites], birds (turkey, duck, cockerel, pheasant, and chicken), amphibians (i.e. Spadefoot toads, and ambystomatid salamanders), fish (i.e. poeciliid fishes), and mammals (rats, mice, and rabbits) (Fox, 1975a; Polis, 1981). Pfennig et al. (1991) and Gould et al. (1980) observed regional differences in the expression of cannibalistic behaviour in tiger salamander larvae (*Ambystoma tigrinum*), and the tobacco budworm (*Heliothis virescens*), respectively.

Hybridization between two species of poeciliid fishes (cannibalistic species *Poeciliopsis monacha* and non-cannibalistic *P. lucida*) produces offspring that exhibit intermediate cannibalistic tendencies between those of the parents (Thibault, 1974). Thibault (1974) observed differences in proportions of conspecifics consumed, from 0.95 in *P. monacha* to 0.0 in *P. lucida*, while proportions of conspecifics consumed by hybrids was 0.74. Subsequent back-crosses between hybrids and original parental species also produced offspring with intermediate cannibalistic tendencies (0.88 when back-crossed with *P. monacha*, and 0.12 when back-crossed with *P. lucida*) (Thibault, 1974). These results suggest cannibalism is the result of polygenic inheritance (Fox, 1975a; Thibault, 1974). Thibault (1974) noted that hunger was less influential to cannibalism than density; individuals starved for 2-4 days did not consume more conspecifics than those that were well-fed.

Nishimura and Isoda (2004) note that cannibalism is an evolutionary stable state and their models suggest that it is a predictable evolutionary consequence of crowding, low availability of alternative food resources, and possibility of starvation. The energetic and nutritional benefits of cannibalism should favour its expression, especially where alternate prey is scarce (Nishimura and Isoda, 2004; Polis, 1981). Cannibalism has been shown to improve growth, development, and/or survivorship in many species, potentially improving the fitness of these individuals (Fox, 1975a; Polis, 1981). In some species, conspecifics may represent an important food source and may comprise a large proportion of the overall volume of prey consumed (Polis, 1981; Pizzatto and Shine, 2008). For example, Dushin (1975) found that tadpoles and smaller conspecifics represent approximately 20% of prey volume consumed by adults of two European frog species, the edible frog (*Pelophylax esculentus*) and the marsh frog (*Pelophylax ridibundus*) (Pizzatto and Shine, 2008). Conspecific prey have been found to comprise up to 7.1%, 22.6%, and 28.4% of the prey by weight consumed by newts (*Notophthalmus sp.*), snakes (*Australeps sp.*), and scorpions (*Smeringurus mesaensis*), respectively, despite the frequency of cannibalism typically being only 0.1%, 2.3%, and 9.1%, respectively, for each of the aforementioned species (Burton, 1977; Polis, 1980, 1981; Shine, 1977). Cannibalism may also act to supplement vital nutrients (e.g. protein, vitamins, lipid, water, etc.) for individuals experiencing nutritional deficiencies [Jefferson et al., 2014 (Chapter 2); Herak and Mitin, 1977; Polis, 1981; Sikand and Renade, 1975]. These benefits may be accrued through direct nutritional benefits, through a reduction in competition and usurpation of resources, and/or reduction in conspecifics producing chemicals that may function to limit growth (Fox, 1975a; Nishimura and Isoda, 2004; Polis, 1981; Thibault, 1974; Rose, 1959).

Interestingly, Nishimura and Isoda (2004) note that their theoretical models of cannibalism as a foraging game suggest cannibalism is only favoured where conspecific prey

are moderately profitable, but not where they represent low or high profit prey. Obviously where conspecific prey represent low profit prey cannibalism would not be beneficial (Nishimura and Isoda, 2004). However, the authors also conclude that cannibalism is not favoured where conspecifics represent a high profit diet because the resulting increase in the rate of cannibalism would decrease the energy acquired from the victim as a result of counter attacks from the potential victims (Nishimura and Isoda, 2004). Nishimura and Isoda (2004) also indicate that as the rate of cannibalism increases among all individuals, expected survival decreases for all individuals across the population. However, the authors go on to state that chances of survival are higher for cannibals than for non-cannibals even where rates of cannibalism is high (Nishimura and Isoda, 2004). Cannibalism also becomes disadvantageous, and will be selected against where aggression becomes so high that a cannibal destroys its own genotype faster than that of its competitors, or reduces its chances of reproduction by eliminating all suitable mates (Fox, 1975a).

Stenseth (1985) concluded that cannibalism could evolve as a result of individual selection even in the absence of extreme scarcity of resources. Furthermore, cannibalism is expected to evolve in stable populations where the young individuals with low reproductive potential are being cannibalized by older individuals with high reproductive potential (Stenseth, 1985). Fox (1975a) notes that cannibalism does not necessarily require large increases to fitness to be selected for. Eickwort (1973) found that “the lower the original fitness of an individual, the smaller the incremental gain necessary to select for increased aggression” as cited by Fox (1975a). Cannibalism is therefore most advantageous to individuals at life stages with the lowest survivorship, i.e. very young individuals (Eickwort, 1973; Fox, 1975a).

Maladaptive cannibalism resulting from stress, accidents, and/or unnatural conditions may also occur, however, Polis (1981) argues that even these events may be genetically



controlled and the result of natural selection [Fox, 1975a; Hrdy, 1979; Jefferson et al., 2014 (Chapter 2); Sherman, 1980; Way, 1966]. For example, Bleakney (1958) observed rampant, incomplete cannibalism among potentially starving wood frog tadpoles occupying a wetland that appeared devoid of food. Polis (1981) noted that euryphagous predators may accidentally consume conspecifics through their natural feeding behaviour. Kishida et al. (2009) found that the frequency of cannibalism among larval salamanders (*Hynobius retardatus*) increased where tadpole prey (*Rana pirica*) exhibit an inducible defensive, “bulgy” morphology (Kishida and Nishimura, 2004). The larval salamanders appear to develop an enlarged head and gape in response to the enlarged “bulgy” morph of their prey (Kishida et al., 2009). However, by developing this larger head/gape morphology salamander larvae not only increase their success of predation upon tadpoles, but they also increase intraspecific predation (Kishida et al., 2009).

Filial cannibalism is fairly common across a diversity of species, including those that exhibit parental care (Bandoli 2002; Bandoli 2006; Evans, 1998; Frommen et al., 2007; Japyassú et al. 2003; Lazzaretto and Salvato, 1992; Lissåker and Svensson, 2008; Main and Bull, 1996). Many teleost fish species are capable of identifying kinship; for example Bandoli (2002; 2006) observed that male spottail darters (*Etheostoma squamiceps*) alter the frequency of egg cannibalism relative to the ratio of sired to adopted offspring (Lissåker and Svensson, 2008). However, male three-spined sticklebacks (*Gasterosteus aculeatus*) will completely cannibalize entire clutches of eggs when foreign eggs are present (Frommen et al., 2007). This species has also been observed to at least partially cannibalize the eggs they were guarding under food-deprived conditions (Mehlis et al., 2009). Lissåker and Svensson, (2008) found that male sand gobies (*Pomatoschistus minutus*) are unable to distinguish related eggs from foreign eggs, and will cannibalize eggs indiscriminately when foreign eggs are present. Similarly, DeWoody et al. (2001) found that nest-guarding male tessellated darters

(*Etheostoma olmstedii*) and two species of sunfish (*Lepomis auritus* and *Lepomis punctatus*) consume their own genetic offspring by using molecular techniques to confirm paternity of the consumed embryos. Dickinson and Weathers (1999) observed that western bluebirds (*Sialia mexicana*) may also consume related offspring.

Interestingly, females in some species of fish, including both three-spined stickleback and sand gobies, prefer to spawn with males guarding nests already containing eggs because males tend to invest more in larger broods and offspring are less likely to be cannibalized in this situation (Lissåker and Svensson, 2008). However, Lissåker and Svensson (2008) suggest that indiscriminate egg cannibalism by male sand gobies where unrelated eggs are present is an adaptive behaviour, and males will not adopt eggs to attract females. Presumably such an adaptation suggests potential benefits to the body condition and overall fitness of these males by not expending time and effort on parental care of offspring with questionable paternity, despite the potential loss of related offspring and mating opportunities (Mehlis et al., 2009). Rohwer (1978) also suggests that males in polygynous species may benefit from consuming the resources females have invested in eggs.

Where food is scarce, the sacrifice of some offspring may benefit the fitness of parent(s) and surviving siblings by providing additional food and reducing demand/competition (Polis, 1981). Mehlis et al. (2009) observed that male three-spined sticklebacks exhibited improved body condition under food-deprived conditions when they cannibalized the eggs they were guarding, and body condition was positively correlated to the number of eggs consumed. Eliminating sick, weak, and/or dying offspring may limit the spread of disease, and also could allow parents to spend more time protecting and investing in surviving offspring (Kaplan and Sherman, 1980; Polis, 1981).

Many species are believed to release chemicals that at high population densities can reduce growth and reproduction of individuals within the population (Rose, 1959; Thibault,

1974). Thibault (1974) and Rose (1959) observed that increasing density of conspecifics in poeciliid fishes will negatively affect the fecundity of individuals within the population. Similar observations have been made for species of bacteria, larval echinoderms, *Daphnia*, and tadpoles (Rose, 1959). Thibault (1974) posits that these chemicals concurrently stimulate an increase cannibalistic behaviour in poeciliid fishes. Moore and McKay (1971) have suggested that in this regard cannibalism may function to control population sizes. Similar assertions have also been made for cannibalism acting as a means of population control in sheep blowfly (*Chrysomya albiceps*), yellow perch (*Perca flavescens*), walleye (*Stizostedion vitreum*), predatory mites (*Blattisocius tarsalis*), the Mediterranean flour moth (*Anagasta kiihniella*), an opisthobranch mollusc (*Navanax inermis*), the backswimmer (*Notonecta hoffmanni*), *Toxorhynchites* and *Megarhinus* mosquitoes, and the predatory rotifer (*Asplanchna sieboldi*) (Fox, 1975a).

Cannibalism among young siblings also appears to be relatively common (Polis, 1981). One explanation for the adaptive significance of sibling cannibalism is that embryonic or newborn victims represent food caches that store energy for their cannibalistic kin and act as a method of transferring maternal nutrients to developing offspring (Polis, 1981). It has been suggested that selection may favour sibling cannibalism where females are unable to partition eggs with sufficient nutrients, or do not provide food to offspring by nourishing the developing offspring (Alexander, 1974; Polis, 1980, 1981; Wourms, 1977). Polis (1981) proposes that “nurse” eggs may represent an extreme case of this strategy, and notes that this strategy has been observed in over 100 species including marine snails, spiders, and numerous species of insects. In utero cannibalism is also proposed by Polis (1981) as an extreme case of a food-caching strategy and has been observed in species of salamanders, and sharks. Additionally, sibling cannibalism acts to adjust large broods to a size that can be

supported by the amount of available food, and the large brood size may then acts as a hedge bet (Alexander, 1974; O'Connor, 1978; Polis, 1981; Stinson, 1980).

Many cannibalistic species exhibit a specialized cannibalistic morphology (Crump, 1992; Nishimura and Isoda, 2004; Wakano et al., 2002). Theoretical studies by Dercole and Rinaldi (2002) and Rudolf et al. (2010) both suggest that cannibalism in a monomorphic population could produce a polymorphic population through evolutionary branching. Pierce et al. (1981) identified differences in allozyme frequencies between typical and cannibal polymorphs of larval tiger salamanders. However, the authors caution these differences do not necessarily contribute to cannibalism, and Polis (1981) suggested the necessity for further research in this area. Indeed, Wakano et al. (2002) notes that studies by Collins and Cheek (1983) and Pfennig and Collins (1993) have shown patterns of growth in larval amphibians to be environmentally controlled, and therefore cannibalistic morphs may arise from phenotypic plasticity. Lannoo et al. (1989) observed an intermediate morphology between the typical and cannibal morphs of larval tiger salamanders. Individuals exhibiting this intermediate form exhibited no genetic or morphological differences (no specialization in head size/shape) with the typical morphs, however, these individuals are cannibalistic (Lannoo et al., 1989). The expression of cannibalism in larvae exhibiting the intermediate form precedes a rapid progression in growth and development, which defines this intermediate form (Lannoo et al., 1989).

Currently, the exact factors influencing the expression of cannibal morphs are unclear (Crump, 1992; Wakano et al., 2002). Wakano et al. (2002) states that the assumption that growth strategies are genetically fixed does not necessarily hold, noting that plasticity is known in amphibian larvae. The authors further suggest that polymorphism should result in the absence of an evolutionary stable strategy (ESS) because where a stable strategy exists all plastic individuals should choose that strategy (Wakano et al., 2002). The authors explain this

by suggesting that if all individuals initially follow a strategy that is not an ESS there must be an alternative strategy that may improve the fitness of some individuals (Wakano et al., 2002). Some individuals will inevitably choose the alternate strategy, and in this will lead to polymorphism within the population (Wakano et al., 2002).

### **1.3 Identifying and Evaluating Risk**

The ability to identify threats can provide species with advanced warning of impending risk, and allow them to respond accordingly (Ferrari et al., 2010). The survival of organisms is dependent on their abilities to detect and respond appropriately to various sources of information (Moir and Weissburg, 2009; Pohnert et al., 2007; Relyea, 2004; Schoeppner and Relyea, 2009). Any given species may improve its survivorship by investing in adaptations to evade or deter predation, to limit exposure to pathogens, to survive inclement environmental conditions, and to improve competitive success (Dawkins and Krebs, 1979; Griffiths, 1997; Kiesecker et al., 1999; Michel, 2012; Relyea, 2002; Relyea and Auld, 2004; Richter-Boix et al., 2007; Wellborn et al., 1996). Various sensory cues are used to track and assess ecological conditions (Petranka and Hayes 1998). Indeed, many species utilize chemical sources of information to identify the origin and degree of potential predation threats and the level of competition (Chivers and Smith, 1998; Ferrari et al., 2010; Michel, 2012; Relyea, 2002).

When animals are captured by predators chemical cues are often released from the tissues of injured prey into the environment. Some of these cues may induce avoidance behaviour in local conspecifics (i.e. reduced activity, shelter seeking, and schooling) and are therefore often referred to as alarm cues (Hagman and Shine, 2008; Moir and Weissburg, 2009; Schoeppner and Relyea, 2009). Individuals identifying alarm cues may also induce the expression of phenotypically plastic morphological traits (e.g. larger bodies, modified

locomotory structures) (Van Buskirk and McCollum, 2000; Kishida and Nishimura, 2004; Relyea, 2004; Richter-Boix et al., 2007; Chivers et al., 2008).

However, risk cues are not limited to those of predation nor are they always transmitted chemically (Hoffman and Pfennig, 1999; Relyea, 2004; Richter-Boix et al., 2007). Visual, auditory and tactile cues also present useful information to individuals, and may induce different responses from those elicited from exposure to chemical cues (Hoffman and Pfennig, 1999). Pond desiccation, crowding, and competition are all also known to provoke specific responses in amphibians (Denver, 2009; Relyea, 2004; Richter-Boix et al., 2007). These responses may also be fine-tuned to respond to the nature and intensity of the threat, and the resource availability of the habitat (Relyea, 2004). The ability to produce context-dependent responses to specific threats can dramatically improve survivorship, while minimizing the incurred cost of the response (Van Buskirk and McCollum, 2000; Richter-Boix et al., 2007). Therefore, an evolutionary pressure may exist towards the expression of rapid and efficient responses to highly variable conditions (Dawkins and Krebs, 1979; Van Buskirk and McCollum, 2000; Relyea and Auld, 2004; Richter-Boix et al., 2007).

#### **1.4 Inducible Responses**

Many species of larval and adult amphibians exhibit cannibalistic behaviour (i.e., Bleakney, 1958; Wildy et al., 1999, Walls et al., 1993). These amphibian species often breed explosively in highly variable ephemeral wetlands where they must develop rapidly, and thus represent ideal models for the study of cannibalism. Additionally, in some highly cannibalistic species, larvae produce distinct trophic polymorphisms (i.e. “cannibal morphs”), which typically feature exaggerated anatomical feeding characteristics (wider heads/mouths and hypertrophied vomerine teeth) relative to typical morphs (Hoffman and Pfennig, 1999;

Maret and Collins, 1997; Michimae and Wakahara, 2001; Sheen and Whiteman, 1998; Walls et al., 1993).

The genetic basis for cannibalism and associated morphological traits has been established through common garden studies by Pfennig et al. (1991), and Parris et al. (2005) identifying regional variation in cannibalism frequency (Bolker et al., 2008). However, the fundamental information of the “underlying genetic architecture” currently appears unclear, and in some instances cannibals and non-cannibals appear genetically identical (Bolker et al., 2008; Lannoo et al., 1989; Pierce et al., 1983). It has been suggested that cannibalistic behavior and associated morphologies are the result of phenotypic plasticity (Bolker et al., 2008; Polis, 1981). This appears supported by Fox (1975a) who stated “cannibalism is a normal response to many environmental factors”. Phenotypic plasticity is the ability of an organism to express one of multiple phenotypes for morphology, physiology and behavior from a single genotype, in response to specific ecological and/or environmental conditions (Scheiner, 1993; West-Eberhard, 1989).

Plasticity provides species with the ability to rapidly respond to a variety of changing conditions (Scheiner and Lyman, 1989). This may provide some degree of flexibility in the induction and reversibility of these traits (Hoverman and Relyea, 2007). Species are known to respond to level of risk they are exposed to with regards to environmental conditions (e.g., growth and development responses to climatic conditions), competition (e.g., altering foraging behaviour, digestive efficiency, resource utilization, etc.), and predation (i.e. inducible defences) (Peacor and Pfister, 2006; Relyea, 2002). Denver and Crespi (2006) noted that tadpoles experience trade-offs associated with phenotypic plasticity, tadpole growth and development exhibit an inverse relationship; therefore accelerated development typically results in decreased growth and smaller post-metamorphic body size and decreased adult survivorship and reproductive capacity.

Phenotypic plasticity resulting from trophic interactions is widely documented in larval amphibians (e.g. Lannoo and Bachmann, 1984; Larson et al., 1999; Loeb et al., 1994; Michimae and Wakahara, 2001; Relyea, 2002; Relyea and Auld, 2004; Van Buskirk and Relyea, 1998; Walls et al., 1993; Wildy et al., 1998). Distinct morphological differences in body size, head size and trophic structures between cannibal and non-cannibal morphs of larval Ambystomatid salamanders have been described by numerous researchers (Larson et al., 1999; Loeb et al., 1994; Michimae and Wakahara, 2001; Walls et al., 1993; Wildy et al., 1998). Trophic polymorphism between cannibals and non-cannibals has also been described in a few species of anurans (i.e. spadefoot toads, *Spea bombifrons*, *S. intermontana*, and *S. multiplicata*) (Pfennig, 1992; Pfennig and Frankino, 1997).

Wood frog tadpoles demonstrate high phenotypic plasticity, which alters aspects of their behaviour and/or morphology and potentially improves survivorship under specific environmental and ecological pressures (Relyea, 2002; Relyea, 2004; Relyea and Auld, 2004; Van Buskirk and Relyea, 1998). Tadpoles will often increase tail growth in response to presence of predatory species, while increased competition has been observed to result in increased body size and intestinal length (Relyea, 2002; Relyea and Auld, 2004).

Cannibalism may result in ecological differentiation between cannibals and non-cannibals by broadening trophic resource utilization, and thus increasing trophic niche width of cannibals relative to non-cannibals (Walls et al., 1993). Walls et al. (1993) indicated that cannibalistic morphology is associated with potentially increased foraging success of gape-limited larval long-toed salamanders, due to the increased size range of prey that may be consumed. Wildy et al. (1998) observed that salamanders fed a diet of conspecifics experienced significantly accelerated growth relative to individuals fed either a diet of tadpoles or a mixed diet of tadpoles and conspecifics. Reilly et al. (1992) observed that individual larval tiger salamanders expressing the cannibalistic morphology had



approximately double the mean capture success of large conspecific prey relative to that of individuals of typical (non-cannibal) morphology (33% vs. 17%).

## **1.5 Model Amphibian Species**

Larvae of biphasic amphibians represent ideal models for study in my research for a number of reasons: 1) their tendency to breed explosively, producing a large number of offspring allows for large sample sizes for testing; 2) most species are relatively small and easily maintained and manipulated in laboratory settings; 3) their rapid growth and development provides rapid results in response to manipulations; 4) their natural history is well understood and documented; 5) all species used are typically non-gregarious and indiscriminate consumers; and 6) they are otherwise commonly used in behavioural and ecological studies, providing a wealth of background information (Babbitt et al., 2003; Denton and Beebee, 1997; Morin, 1983a; 1986; 1987; 1989; Morin et al., 1990).

### **1.5.1 Wood Frogs**

Wood frogs (*Lithobates sylvaticus*) are a species of the family Ranidae, the “true frogs”. Wood frogs have a natural range from the southern Appalachians north through the Maritime Provinces to the treeline of Labrador and northwest to Yukon and Alaska, though are absent from short-grass prairies of Alberta and Saskatchewan, and from central and southern BC (Gilhen, 1984; Stebbins, 2003). Additionally, this species exhibits the most northern range of any North American herpetofauna (Gilhen, 1984; Stebbins, 2003). In the eastern portion of their range, adults typically occupy shady, moist forest, while in the northwest they often occupy meadows with proximal tree stands, and/or ponds (Stebbins, 2003). In Canada, wood frogs are typically the first amphibian species to begin breeding,

often moving to natal wetlands as soon as the ice begins to melt (Gilhen, 1984; Stebbins, 2003).

Wood frogs are biphasic, and are primarily limited to breeding in temporary wetlands because of the vulnerability of their offspring to predation by large vertebrate predators (i.e., they lack unpalatable chemicals in their skin); ephemeral wetlands naturally exclude predatory vertebrates such as fish or newts that can decimate larval populations (Cortwright and Nelson, 1990; Griffiths, 1997; Szuroczki and Richardson, 2011; Wellborn et al., 1996). Adults breed explosively and synchronously, with breeding often completed over a period of approximately a week within a given wetland; females produce globular egg clutches attached to submerged vegetation and may contain as many as 500 to 3000 eggs (Gilhen, 1984; Duellman and Traub, 1986). Wood frogs exhibit strong breeding site fidelity with less than 20% of surviving metamorphs permanently dispersing away from natal wetlands (Berven and Grudzien, 1990). Tadpoles follow the larval development documented by Gosner (1960), consisting of 46 developmental stages from fertilization to completion of metamorphosis. Development of tadpoles to metamorphosis is typically achieved within two months (Herreid II and Kinney, 1967).

Wood frog tadpoles are non-gregarious and typically only aggregate where densities are high or are forced to congregate around resources (Waldman, 1985; Wells, 2007). Larvae are primarily considered generalist consumers, though they have been noted to consume a relatively high proportion of invertebrate prey (Petranka and Thomas, 1995; Quammen and Durtsche, 2003). Schiesari et al. (2009) even suggest wood frog tadpoles are potentially primary predators in some ponds, and they achieve this despite a lack of predatory adaptations (i.e. enlarged jaws, modified beaks and teeth, and shortened digestive tracts) (Crump, 1983; Petranka and Thomas, 1995; Wassersug, 1980; Wassersug et al., 1981). Numerous studies have implicated wood frogs as active predators of heterospecific

amphibian eggs and larvae (i.e., Burley et al., 2006; Petranka et al., 1994). Morin (1983) noted that red-spotted newts are the only species as effective at amphibian egg predation as wood frog tadpoles. Bleakney (1958) has also described wood frog tadpoles as facultative cannibals.

### **1.5.2 Ambystomatid Salamanders**

Mole salamanders (family Ambystomatidae) are aptly named for the tendency of adults of many species to occupy the underground tunnels and dens produced by burrowing animals (Gilhen, 1984). These salamanders are typically biphasic, although metamorphosis is not obligatory in all individuals or species (e.g. axolotl, *Ambystoma mexicanum*) (Petranka, 1998; Pough et al., 2004). Many species of larval ambystomatids are generally non-gregarious with aggregations often attributed to limitation of spatial and/or dietary resources, however, social behaviour has been observed in some instances (Crane et al., 2012; Duellman and Trueb, 1986; Petranka, 1998). Species are highly predatory, with some species exhibiting cannibalistic behaviour (Cortwright and Nelson, 1990; Petranka, 1998; Walls et al., 1993; Wildy et al., 2001). Distinct morphologies have been described in many species and have been associated with cannibalistic behaviour; “cannibal morphs” typically feature exaggerated anatomical feeding characteristics (wider head/gape and hypertrophied vomerine teeth) relative to typical morphs (Hoffman and Pfennig, 1999; Maret and Collins, 1997; Michimae and Wakahara, 2001; Sheen and Whiteman, 1998; Walls et al., 1993). Agonistic behaviour is common among larvae in most species, and can commonly result in partial cannibalism through biting of gills, tails, and/or limbs (Petranka, 1998; Wildy, et al., 2001).

#### *Long-Toed Salamanders*

This research utilizes a sub-species of larval long-toed salamanders (*Ambystoma macrodactylum columbianum*), from the Cascades mountain region of Oregon, and which has a natural distribution from southeastern Alaska and northern BC, through west-central Oregon, and west through the western half of northern Idaho (Petranka, 1998; Wildy et al., 1998). Adults breed synchronously in a variety of wetlands from temporary ponds to lakes, with females producing egg masses that contain an average of 15–17 eggs (Howard and Wallace, 1985). Larval period varies from 3–26 months based on elevation and duration of the wetland. Larvae feed primarily upon a variety of aquatic invertebrates, and tadpoles of the Pacific tree frog (*Pseudacris regilla*). While, small larvae are dependent upon sit and wait predation, larger larvae may actively stalk their prey along pond bottoms (Petranka, 1998). Trophic polymorphism is well documented in this species, and has been suggested to facilitate cannibalism among similar size conspecifics (Anderson, 1967; Petranka, 1998; Walls et al., 1993; Wildy et al., 1998). However, in locations where larvae develop over multiple years before metamorphosing, larger established larvae may also feed upon smaller cohorts (Petranka, 1998).

### *Ringed Salamanders*

Larval ringed salamanders (*A. annulatum*) collected from central Missouri are also used in this research. This species has a natural distribution through central Missouri, south through northwestern and central Arkansas into eastern Oklahoma. Adults breed explosively within temporary wetlands over a prolonged 2–3 month period (Petranka, 1998). Females produce strings of eggs that may hold 50 or more eggs and average densities as high as 638eggs/m<sup>2</sup> have observed in wetlands (Peterson et al., 1992; Petranka, 1998). Larval development begins in the fall and progresses through the winter, requiring 6–8.5 months to reach metamorphosis (Hutcherson et al., 1989). Larvae in the collection pond in Missouri

primarily feed upon aquatic invertebrates, but may also consume frog eggs, earthworms, and conspecifics (Nyman et al., 1993). Cannibalism occurs as a result of the inherent size variation within the population, however different head morphologies have also been observed within the population (Nyman et al., 1993).

## **1.6 Objectives:**

The overall objective of this research was to examine the proximal mechanisms that influence cannibalism within larval amphibian populations. This thesis explores conditions that facilitate and mediate cannibalistic behaviour through the culmination of seven laboratory experiments and studies of natural populations discussed across five chapters:

### **1. Relative nutritional value of cannibalism and the influence of nutritional stress.**

If individuals vigorously compete to acquire profitable diets, it stands to reason that a high quality diet may be highly sought after, especially in conditions of limited resources and a drying habitat. In Chapter 2, I examined whether cannibalism is indeed a high quality diet. If it is ideal then cannibalism may serve as a self-perpetuating mechanism, providing a potentially abundant and highly profitable diet. To achieve this I tested wood frog tadpoles for differences in growth and development between tadpoles fed a diet of conspecific tissues and those fed a variety of alternative diets varying in nutritional quality. If conspecific tissues do represent an ideal diet, then tadpoles fed this diet should exhibit equal or greater growth and development relative to those fed a high quality diet of similar protein content. Additionally, starvation and low quality diet treatments were included in experiments to a) act as a lower end measure for efficacy of cannibalism as a diet; and b) to provide information regarding the hormonal response of tadpoles to nutritional stress, and whether this may influence individuals to become cannibalistic. I expected that conspecific tissues

would be more beneficial to tadpole growth and development relative to both starvation, and a low quality diet. Furthermore, I predicted that tadpoles would exhibit elevated corticosterone levels in response to nutritional stress from both starvation and reliance upon a low quality diet. Corticosterone acts as an appetite stimulant in tadpoles and could cause tadpoles to become increasingly aggressive in their feeding behaviour, potentially leading to cannibalism. Additionally, the coefficient of variation in mass of tadpoles was used to assess differences in the degree of competition tadpoles encounter among diets. Large variation in mass among individuals should be indicative of high competition, with those individuals expressing traits that provide them a competitive advantage outgrowing less competitive individuals (Peacor and Pfister, 2006). I expected that tadpoles would exhibit the greatest competition for the high quality diet due to their expected preference for this diet. Intense competition for a high quality diet could result increasingly aggressive behaviour and potentially lead to cannibalism.

## **2. Do chemical cues released from conspecific tissues influence cannibalistic behaviour?**

In Chapter 3, I explored whether or not chemical alarm cues released from macerated conspecific tissues would deter tadpoles from feeding when they were added to a laboratory diet. The purpose of this was to test observations from Chapter 2, where tadpoles fed conspecific tissues appeared to preferentially avoid feeding upon this diet for approximately two weeks. If alarm cues do deter tadpoles from feeding, it might explain the results observed in Chapter 2 and would represent an innate mechanism deterring cannibalistic behaviour. In this experiment I tested wood frog tadpoles for differences in growth, development, and morphology between those fed a diet soaked in alarm cues and those fed a diet soaked in de-chlorinated water. If alarm cues deter tadpoles from feeding then those exposed to these cues should exhibit slower growth and development, relative to those in the control group.

### **3. How do competition, dietary cues, and timing influence feeding behaviour?**

In Chapter 4, I tested the influence of competition and dietary cues on tadpoles' feeding response, and I examined this across multiple observational periods to determine if or how these responses changed over time. The purpose here was to further expand upon concepts explored in the previous chapters two and three, characterizing how these variables interact to influence tadpole feeding behaviour. To accomplish this I raised tadpoles in groups alternating their diet between a high and low quality to acclimate them to each. At specific time points tadpoles were removed from stock tanks and tested for their feeding initiation times for the high and low quality diets, a diet of conspecific tissues alone, and conspecific tissues soaked in chemical cues of the high or low quality diets. This was tested with individual and pairs of tadpoles to test the effect of competition on feeding initiation times for each diet. If competition does indeed influence tadpole feeding, their initiation times should be reduced in the presence of a competitor. Similarly, if tadpoles discriminate among diets they should exhibit preferences through differences in feeding initiation times, and if they do exhibit dietary preferences the addition of dietary cues may alter tadpole response to conspecific tissues.

### **4. Is there a functional link between polymorphism and cannibalism in natural populations of larval salamanders?**

In Chapter 5, I examined the putative link between trophic polymorphism and cannibalism within a natural population of larval long-toed and ringed salamanders. The expression of the "cannibal" head morphology is suggested to facilitate cannibalism among larvae, which due to the concurrent nature of adult breeding are all of similar age and size within a given wetland. To determine if this occurs in natural populations specimens

collected from a natural population known to exhibit polymorphisms were analyzed for differences in head morphology and C and N stable isotope values to identify whether differences in head morphology correspond with differences in diet. If head morphology is strongly associated with cannibalism I expect “cannibal morphs” to occupy a higher trophic position relative to “typical morphs”, and that “typical morphs” will represent a potential diet for “cannibal morphs” based on stable isotope data. In long-toed salamanders it is expected that head morphology will be necessary to facilitate cannibalism due to their synchronous breeding, and thus lack of inherent size differentiation within the population. Conversely, due to their staggered breeding cannibalism is expected to occur among larval ringed salamanders irrespective of head morphology, but may be more prevalent when individuals exhibit the larger head morphology (“cannibal morph”).

## **1.7 Significance**

The research presented in this thesis explores the roles of some influential mechanisms in the expression of cannibalistic behaviour. Although many of the aspects covered in this thesis have been covered in previous research (i.e. diet, morphology) I have attempted to take novel approaches in testing these concepts (Nyman et al., 1993; Petranka and Thomas, 1995; Pierce et al., 1983; Wildy et al., 1998; etc.). I believe the results of this research provide a more well-rounded understanding of cannibalism, its evolution, and its potential influence on population dynamics.

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## **Chapter 2: Frugal cannibals: how consuming conspecific tissues can provide conditional benefits to wood frog tadpoles (*Lithobates sylvaticus*).**

Contents of Chapter 2 have been published in *Naturwissenschaften* (2014) Volume **101**: 291-303 under joint authorship of Keith A. Hobson, Brandon S. Demuth, Maud C.O. Ferrari, and Douglas P. Chivers. Brandon Demuth conducted corticosterone analysis. The original manuscript was modified to reflect the comments of the examining committee, and the overall formatting of the thesis. Additionally, supplementary resources that were included as online only materials in the published manuscript (i.e. corticosterone and stable isotope analysis methods, and figures 2.2, 2.4, and 2.5) have been included within the methods and results sections.

### **2.1 Abstract**

Tadpoles show considerable behavioural plasticity. When population densities become high, tadpoles often become cannibalistic, likely in response to intense competition. Conspecific tissues are potentially an ideal diet by composition and should greatly improve growth and development. However, the potential release of alarm cues from the tissues of injured conspecifics may act to deter potential cannibals from feeding. I conducted multiple feeding experiments to test the relative effects that a diet of conspecifics has on tadpole growth and development. Results indicate that while conspecific tissues represent an alternative to starvation and provide some benefits over low protein diets, such a diet can have detrimental effects to tadpole growth and/or development relative to diets of similar protein content. Additionally, tadpoles raised individually appear to avoid consuming conspecific tissues, and may continue to do so until they suffer from the effects of starvation. However, tadpoles



readily fed upon conspecific tissues immediately when raised with competitors. These results suggest that cannibalism may occur as a result of competition, rather than the specific quality of available diets, unless such diets lead to starvation.

## **2.2 Introduction**

Many species of biphasic amphibians rely on ephemeral wetlands for larval development (Wellborn et al., 1996). The unpredictable conditions and duration of such habitats require inherent plasticity in behaviour, growth and development that are necessary for rapid adaptive responses to local conditions (Relyea 2004; Richter-Boix et al. 2007). Pond drying increases the risk of desiccation for developing tadpoles and can simultaneously increase population densities while reducing dietary resource availability, thus increasing competition (Babbitt and Meshaka 2000; Newman 1987). Larval amphibians experiencing such conditions should increase their rate of development to rapidly achieve metamorphosis and escape the drying habitat (Wellborn et al. 1996).

Deteriorating conditions associated with drying ephemeral wetlands can lead to reductions in quality and/or quantity of dietary resources (Babbitt and Meshaka 2000). Under such conditions, altering diet selection may improve growth and development. In particular, cannibalism can improve survivorship of individuals within the population (Fox 1975). Cannibalism is both an intraspecific predator-prey interaction, and a form of interference competition, “limiting population size before the resource itself becomes limiting” (Fox 1975). Conspecific tissues represent a potentially ideal diet satisfying all nutritional requirements for growth, development, and reproduction because all nutrients should be available in the appropriate proportions (Meffe and Crump 1987). Cannibalism may sustain individuals to metamorphosis under conditions of dietary stress (Bleakney 1958). Even in the absence of severe dietary stress, cannibalism may be highly advantageous from a growth

perspective, so long as the costs of cannibalism do not exceed the benefits. Cannibalism may reduce fitness of individuals through increased risk of injury from retaliation (Wildy et al. 2001), increased exposure to pathogens (Bolker et al. 2008), and reduced survivorship of kin (Fox 1975). Additionally, species of *Ceratophrys* and *Gephyromantis* tadpoles appear to have evolved acoustic signals that may also reduce the occurrence of cannibalism among conspecifics (Costa et al. 2014; Reeve et al. 2011)

Petranka and Thomas (1995) suggest that the evolution of explosive concurrent breeding among wood frogs (*Lithobates sylvaticus*) was influenced by the efficiency of tadpole cannibalization of vulnerable conspecifics. Indeed, synchronized explosive breeding can reduce the risk of cannibalism by limiting the differences in size and development among individuals (Crossland et al. 2011; Petranka and Thomas 1995). Additionally, food availability and conspecific density influence the expression of intraspecific aggression and cannibalistic behaviour (e.g. Collins and Cheek 1983; Wildy et al. 2001). Conversely, Babbitt and Meshaka (2000) suggest that cannibalism among free-feeding tadpoles is an adaptation to low quality and/or abundance of food rather than to pond drying, due to the observation of prolonged larval development among tadpoles fed a high-quality diet and conspecific tissues.

Individuals engaged in cannibalism are often older tadpoles that attack eggs and newly hatched larvae (Petranka and Thomas 1995). It is unclear why cannibalism is not more common and pervasive throughout tadpole populations. The objective of my study was to further explore the efficacy of cannibalism as a diet, and characterize mechanisms that may mediate its emergence within populations.

An initial experiment was developed to compare the relative efficacy of conspecific tissues as a diet, to that of a high protein diet, on individual tadpoles. Previous studies suggest that tadpole growth and development is protein limited (McCallum and Trauth 2002; Kupferberg 1997; Steinwascher and Travis 1983). Therefore, I used protein as a simple

measure of quality among diets. I expected, based on the assumption that cannibalism represents an inherently suitable diet by composition that a diet of conspecific tissues should facilitate equal or greater tadpole growth and development relative to that of tadpoles fed a diet of similar protein content (Meffe and Crump 1987).

Subsequently, a second experiment was conducted to further explore cannibalism as a diet for larval wood frogs. In this experiment tadpoles were raised in groups, subjecting them to relatively high intraspecific competition. High quality, low quality, mixed (high and low), and starved dietary treatments were included to further examine the relative efficacy of conspecific tissues as a diet. Cannibalism is often observed in situations of high conspecific density, therefore I expected that tadpoles raised in a competitive environment would readily consume conspecific tissues [Jefferson et al. 2014 (Chapter 4)]. Richter-Boix et al. (2007) observed that tadpoles may preferentially consume dietary resources that are ideal to facilitate growth or development in response to predation or pond drying, respectively. Additionally, numerous studies have observed the importance of animal tissues in the diet of wood frog tadpoles. I expect that tadpoles fed the mixed diet would preferentially consume the high quality dietary component over the low quality component. Starvation and/or low protein dietary treatments may induce cannibalistic behavior in tadpoles due to the nutritional stress that may result from these treatments (i.e. Venesky et al., 2012). Crespi and Denver (2005) observed that starvation stimulated increased whole body corticosterone content in pre-metamorphic Western spadefoot toad (*Spea hammondi*) tadpoles. They indicated that secretion of corticosterone induces foraging through appetite stimulation, facilitating the restoration of energy reserves during stress-induced anorexia. Similarly, Venesky et al. (2012) suggest that tadpoles experiencing nutritional deficits from consumption of a primarily low-protein content diet can lead to elevated corticosterone levels. Therefore, I expected tadpoles suffering nutritional deficiencies to exhibit elevated corticosterone levels.

Under such conditions, elevated corticosterone levels could encourage cannibalistic behaviour.

A third experiment was conducted to test whether the effects of a cannibalistic diet would persist if tadpoles were tested at later stages of development after being fed a control diet composed of low and high protein diets for approximately a month prior to testing. I also included two treatments where conspecific tissues were combined equally by mass with the high and low protein diets to test how tadpole growth and development was affected. It was predicted that, due to the protein content of conspecific tissues, tadpoles fed a mixture of cornmeal and conspecific tissues would exhibit improved growth and development relative to those fed cornmeal alone, while those fed conspecific tissues and brine shrimp would do poorly relative to individuals fed brine shrimp alone.

## **2.3 Materials and Methods**

### **2.3.1 Collection and Maintenance**

All collections were conducted at a series of disjunct ponds within Cranberry Flats Conservation Area flood plain in Saskatoon, SK (N 52°01.824', W 106°42.403'), and from temporary roadside ponds near Dalmany, SK (N 52°17.368', W 106°52.639'), Canada. All specimens were transported to the University of Saskatchewan, and maintained in multiple 38L glass stock aquaria filled with de-chlorinated water and raised to stage 25 (Gosner 1960). Tadpoles were randomly selected for experimental treatments at free-feeding stage 25.

Collection sites were open canopy and contained semi-aquatic or biphasic species of predatory invertebrates (e.g. odonates and dytiscids). Vertebrate predators (e.g. garter snakes, adult frogs, and predatory birds and mammals) are common and represent a potential threat to tadpoles.

Experiments were performed under a controlled 14:10 h light:dark photoperiod. All tadpoles received 100% water changes three days a week (M, W, F) and were fed immediately after all water changes were completed, and were fed *ad libitum*. Water quality (i.e. pH,  $\text{NH}_3$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ) of experimental containers was assessed a minimum of once a week within 24 h of a feeding using commercially available aquarium test strips. Mean ( $\pm$  SE) water temperatures for experimental containers were 17.1° C ( $\pm$  0.6) through the course of the experiments. Tadpoles were stocked at a density of one tadpole  $\text{L}^{-1}$  in each experiment to reduce differences in potential informative cue (i.e. alarm cues or competitive cues) concentrations that could influence tadpole behavior (Relyea 2002).

### **2.3.2 Experimental Procedures**

#### *Experiment 1*

When tadpoles had achieved approximately stage 25, 90 tadpoles were randomly selected for testing, divided into two groups of 45 and randomly assigned one of two experimental treatments. Initial masses and Gosner stages were recorded for every individual. Five tadpoles were randomly selected and euthanized immediately using a buffered tricaine methansulfonate (TMS) solution. The masses and developmental stages of these euthanized tadpoles were recorded and prepared for stable isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) analysis. The stable isotope values of these individuals were also used as the initial values of tadpoles in Experiment 2.

Tadpoles were individually housed in 1L plastic containers filled with de-chlorinated water. Tadpoles were individually housed to remove any potential competitive and predatory interactions between individuals. Tadpoles were fed either a commercial frozen brine shrimp (BS) diet, or a cannibalistic diet of homogenized whole body conspecific tissues (CAN).

Frozen BS was chosen because of its relatively high protein content. Dry protein of diets was

calculated by multiplying the %N values of dietary samples by 6.25 as described by Sterner and Elser (2002).

To assess relative dietary quality, an independent two-sample *t*-test was used to compare values of estimated final % dry protein compositions between diets. Bonferroni corrected one-sample *t*-tests were used to compare estimated protein composition of experimental diets to mean observed protein values of diets in prior studies. No significant differences were observed in protein composition between BS and CAN ( $t_{[5.6]} = 0.004$ ;  $P = 0.99$ ). Mean ( $\pm$  SE) dry protein % of frozen BS was estimated to be 48.7 ( $\pm$  1.4)%, and that of CAN was 48.7 ( $\pm$  5.9). Protein composition for both diets was significantly greater than the 33% protein of ingested diets of wood frog tadpoles from natural populations reported by Schiesari et al. (2009) (BS:  $t_{[5]} = 10.9$ ;  $P < 0.001$ ; CAN:  $t_{[5]} = 2.6$ ;  $P = 0.04$ ). Mean % protein values of BS were significantly higher than the 44% protein composition of the most profitable diet (100% soybean) tested by McCallum and Trauth (2002) ( $t_{[5]} = 3.3$ ;  $P = 0.02$ ). However, CAN were not significantly different from the 100% soybean diet ( $t_{[5]} = 0.8$ ;  $P = 0.5$ ).

The CAN diets were produced by collecting wild tadpoles from the same ponds as tadpoles used in the experiment. Initial tadpoles used to produce the CAN diet were gut loaded by briefly feeding frozen BS prior to being homogenized with an ultrasonic homogenizer. Additional tadpoles were collected for feeding at days 21, 28 and 42 but were not fed in the lab. Randomly selected tadpoles from these additional collections were used to provide mass and developmental values of wild tadpoles throughout the study. No tadpoles could be collected after day 42 due to flooding of the sampling site from the adjacent Saskatchewan River.

Frozen foods were weighed and thawed in 200mL of de-chlorinated water and distributed among tadpoles in respective treatments. Although tadpoles were fed *ad libitum*,

food rations were adjusted reflecting observed feeding behaviour to allow individuals to feed to satiation over the 48h feeding period and minimize water fouling.

Groups of up to five tadpoles were randomly selected at the end of each week, for nine weeks, and were euthanized. Wet masses and developmental stages of euthanized tadpoles were recorded. Results from mortalities were excluded from this study because tissue degradation of deceased specimens could have led to erroneous measurements. Snout-vent length (SVL), mass, and developmental stages of randomly selected wild tadpoles were recorded when additional specimens were collected for cannibal diet preparation. Experimental tadpoles and their diets were also analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

## *Experiment 2*

At stage 25, tadpoles were randomly distributed among 40, 10L experimental aquaria; five treatments with eight replicates per treatment, and a stocking density of 10 tadpoles per aquarium (one tadpole  $\text{L}^{-1}$ ). Tadpoles were randomly assigned to one of five experimental dietary treatments: 1) frozen brine shrimp (BS); 2) cornmeal (CM); 3) mixed 50% brine shrimp and 50% cornmeal (BS/CM); 4) homogenized conspecifics (CAN); 5) starvation (ST).

As in Experiment 1, estimates of dry protein content of diets were calculated by multiplying the %N values of dietary samples by 6.25 as described by Sterner and Elser (2002). Frozen BS was again specifically chosen because of its relatively high protein. In this experiment the estimated mean ( $\pm$  SE) dry protein content of BS was  $54.9 (\pm 1.6)\%$ , and protein content of CAN was  $53.6 (\pm 1.6)\%$ . A diet of CM was selected because of its relatively low protein content (mean  $\pm$  SE:  $6.6 \pm 0.2\%$ ) and was identified by McCallum and Trauth (2002) as a low quality diet for wood frog tadpoles. An ANOVA used to compare protein content among BS, CM and CAN found that there was a significant difference in

estimated protein content among diets ( $F_{[2,30]} = 109.7$ ;  $P < 0.001$ ). *Post hoc* Tukey pairwise comparisons indicated that there was no significant difference between BS and CAN ( $P = 0.9$ ), and both BS and CAN exhibited significantly higher estimated dry protein content relative to CM (both  $P < 0.001$ ). An ANOVA test of estimated dry protein content among BS and CAN between Experiments 1 and 2 identified no significant differences ( $F_{[3,33]} = 1.02$ ;  $P = 0.4$ ).

Tadpoles used to produce the cannibal diet were coarsely homogenized with an ultrasonic homogenizer. All tadpoles used to produce the cannibal diet were raised on a mixed diet of frozen BS and CM. These tadpoles were never fed within 48h of being euthanized to produce the conspecific diet to reduce the effects that chemical cues from these fresh diets may have had on test specimens.

The experiment was concluded after 14d (to accommodate starved tadpoles) and all surviving tadpoles were euthanized using a buffered TMS solution. SVL, mass, and developmental stages of tadpoles were recorded immediately after being euthanized.

Two tadpoles in each replicate of each treatment (where tadpole survival allowed) were randomly selected and analyzed for total body corticosterone concentration. Analysis of whole body corticosterone was used in the second experiment to determine if the low quality and cannibalistic diets could illicit hormonal responses that may alter corticosterone levels.

Subsequently, up to three tadpoles were randomly selected from all treatment replicates (where survival allowed) and a minimum of eight replicates of each diet (BS, CM, and CAN) were analyzed for C and N stable isotope composition. Tissue values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were used as a tool to assess dietary composition (i.e. protein and lipid content) as well as to track tadpole foraging. The stable isotope values allowed us to assess assimilation of dietary resources in each of the experiments.



### *Experiment 3*

Tadpoles were maintained in four 38L glass aquaria filled with de-chlorinated water. Tadpoles in all stock aquaria were maintained at approximately the same densities, and all were within the range of natural population densities observed by Biesterfeld et al. (1993). When the majority of tadpoles in these tanks had achieved stage 29 of development individuals were haphazardly removed from stock aquaria and randomly distributed among 48, 10L experimental aquaria at an initial stocking density of 10 tadpoles per aquarium (one tadpole L<sup>-1</sup>). Each aquarium was filled with de-chlorinated water in advance and had been assigned to one of six dietary treatments, with eight replicates per treatment. The six experimental dietary treatments were: 1) frozen brine shrimp (BS); 2) cornmeal (CM); 3) mixed 50% brine shrimp and 50% cornmeal (BS/CM) by mass; 4) homogenized conspecifics (CAN); 5) mixed 50% brine shrimp and 50% conspecific tissues (BS/CAN) by mass; and 6) mixed 50% cornmeal and 50% conspecific tissues (CM/CAN) by mass. Tadpoles were fed BS/CM in the 10L experimental aquaria for two weeks until the tadpoles in each tank reached a minimum developmental stage of 29. The experiment was run for 14d to be consistent and comparable with Experiment 2.

Due to tadpole mortalities prior to the initiation of testing, the number of individuals in each tank at the start of testing was reduced to eight tadpoles per aquaria across all treatments. SVL, developmental stage, and mortalities were recorded at the end of the 14d testing period; body mass was not be obtained for tadpoles in this experiment.

#### **2.3.3 Stable Isotope Analysis**

Tadpole specimens were freeze-dried in a Labconco Corp. Freezone® freeze drier for approximately 24h. Freeze-dried whole body tadpoles were pulverized to a fine powder, weighed and packaged at the National Hydrology Research Center of Environment (NHRC),

Saskatoon, SK, Canada. Homogenized whole body samples were used to compensate for differences in fractionation between animal tissues (Biasatti 2004). Dry powder samples were packaged in ~ 0.1mg portions, using Elemental Microanalysis Ltd. 5 x 3.5 mm tin capsules. Samples were subsequently submitted for C and N stable isotope mass spectrometry analysis to the Stable Isotope Hydrology and Ecology Research Laboratory at NHRC, and the Stable Isotope Laboratory of the Department of Soil Science, University of Saskatchewan. Previous lipid proportions of wild wood frog tadpoles were consistently below 5%. Therefore, no lipid processing of samples submitted for isotope analysis was warranted (Post et al. 2007). Values of stable isotopes ( $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ ) were found as the deviance ( $\delta$ : delta) of the ratio of heavy to light isotopes (i.e.,  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ) within a sample, to that of an international standard, and expressed in parts per thousand (‰) (Biasatti 2004). Measurement errors of  $\pm 0.3\text{‰}$  and  $\pm 0.5\text{‰}$  (for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively) were observed, using replicates of two internal laboratory standards (albumen and bowhead whale baleen). Discrimination factors of stable isotopic values were calculated as the difference in isotopic values between diet and consumer ( $\Delta X = \delta X_d - \delta X_c$ ). Stable isotope values were quantified and reported as described in Jardine et al. (2003).

#### **2.3.4 Corticosterone Analysis**

Tissue preparation and corticosterone quantification was a modified protocol from Thomas and Janz (2011). Frozen tadpoles were thawed on ice and homogenized in sodium buffer containing gelatin (pH 7.6) using a Tissue Tearor (Fisher Scientific, Houston, TX, USA). Whole-body homogenate of tadpoles originating from the same treatment tanks were pooled and extracted with diethyl ether, evaporated under a stream of nitrogen, and reconstituted in a sodium phosphate buffer. Extracted samples were stored at  $-80^\circ\text{C}$  until corticosterone analysis.

Corticosterone was determined using an enzyme-linked immunosorbant assay (ELISA) kit (Oxford Biomedical Research, Oxford, MI, USA). Corticosterone concentrations were quantified using a SpectraMAX 190 (Molecular Devices Corp., Sunnyvale, CA, USA). Extracted samples were run in duplicate and the mean results with < 15% coefficient of variation were used for data analysis.

### **2.3.5 Statistical Analyses**

#### *Experiment 1*

A one-way multivariate analysis of variance (MANOVA) was used to compare final values of SVL, mass, and developmental stages between tadpoles fed the BS diet, and those fed the CAN diet. To adjust for differences in developmental stages, this same procedure was used to compare the masses of tadpoles at comparable stages of development (between stages 27-32).

Overall values of SVL, mass, and developmental progression were compared among wild, BS fed, and CAN fed tadpoles at days 21, 28, and 42 (days where data existed for all three groups) using a one-way MANOVA; all paired comparisons were made using Hotellings T-square test. Overall differences in discrimination factors of tadpoles were compared using an ANOVA. Pairwise comparisons were performed using Tukey's Honestly Significant Difference (HSD) test for differences in discrimination factors of tadpoles between days zero and 14 fed each of the experimental diets, and with tadpoles fed CAN between days 28 and 63. Similarly, one sample *t*-tests were used to assess the difference of these discrimination factors from the expected mean discrimination factor of 1.0‰, over the same time period.

## *Experiment 2*

All statistical analyses were performed on mean data pooled from within each individual aquarium. Multiple one-way ANOVAs were used to compare mortalities, final SVL, mass, development (final Gosner stage), and whole body corticosterone levels of tadpoles among treatments. Additionally, differences in intra-population growth were analyzed by performing a one-way ANOVA on the coefficient of variance (CV) of tadpole mass among all treatments except ST. Tukey HSD tests were subsequently used to conduct pairwise comparisons among treatments where ANOVA tests indicated overall significant differences.

$\delta^{13}\text{C}$  values of consumers are typically similar to those of their diet, conversely  $\delta^{15}\text{N}$  values of consumers are typically greater than those of their diet. Therefore, the relative trophic positioning of starved individuals, based on isotopic values relative to those of initial tadpole values, were analyzed using two two-sample *t*-tests with Bonferroni correction. Proportional contributions of BS and CM were estimated using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and the stable isotope mixing model SIAR (R Development Core Team 2010). Mean ( $\pm$  SD) isotopic correction values of 1.0 ( $\pm$  1.2) ‰ and -5.7 ( $\pm$  2.2) ‰ in  $\delta^{13}\text{C}$  were applied for brine shrimp and cornmeal respectively, and 1.1 ( $\pm$  1.9) ‰ and 2.7 ( $\pm$  1.5) ‰ in  $\delta^{15}\text{N}$  for brine shrimp and cornmeal respectively to calculating estimates for mixing models.

## *Experiment 3*

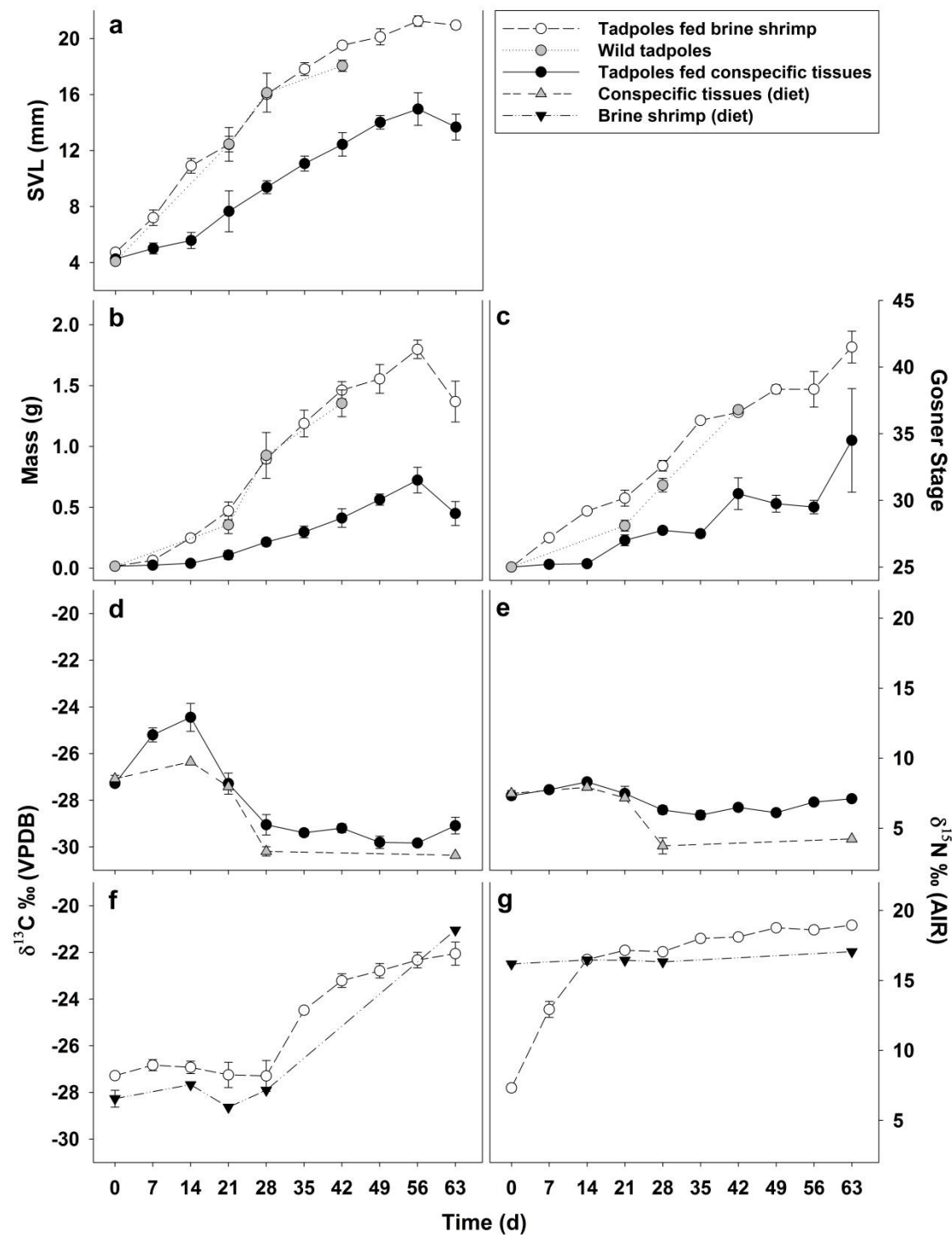
Tadpole SVL, developmental stage, and mortalities were tested using three, one-way ANOVA tests. *Post hoc* Tukey HSD tests were used to make pairwise comparisons among tadpoles in each feeding treatment.

Analyses in all experiments, other than those using SIAR, were performed using Systat (Wilkinson 1998). Outliers were removed where identified through the statistical software. A conservative testing procedure was adopted independently for each experiment by adjusting significance levels using the Holm-Bonferroni correction (Marcus et al. 1976), to reduce the risk of committing a type one error (Sokal and Rohlf 1995). All figures were produced using SigmaPlot (Systat Software, San Jose, CA, USA).

## **2.4 Results**

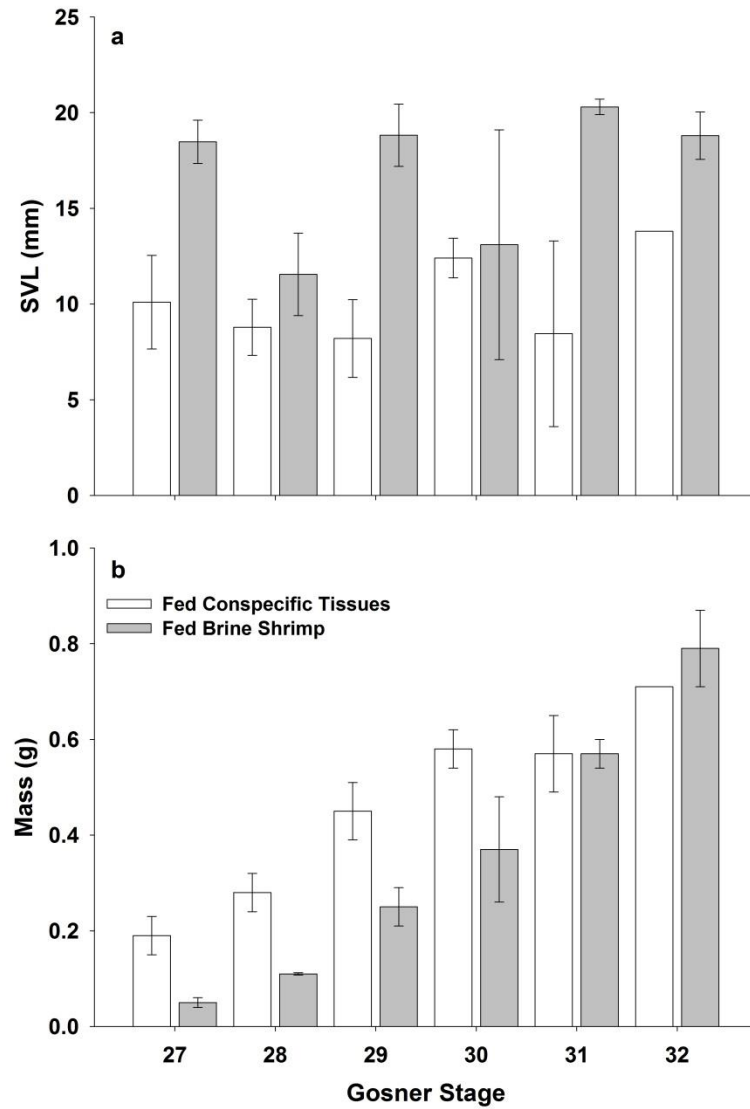
### **2.4.1 Experiment 1**

Tadpoles fed CAN and BS diets had mean ( $\pm$  SE) initial SVL of 4.3 ( $\pm$  0.1) mm and 4.7 ( $\pm$  0.1) mm respectively; tadpoles in both dietary treatments had initial masses of 0.02 ( $\pm$  0.00) g; and initial developmental stages of 25.3 ( $\pm$  0.1) and 25.7 ( $\pm$  0.1) respectively (Fig. 2.1a,b). Wild tadpoles euthanized at day zero had mean ( $\pm$  SE) initial SVL of 4.1 ( $\pm$  0.2) mm; initial mass of 0.02 ( $\pm$  0.00) g; and initial developmental stage of 25.0 ( $\pm$  0.0) (Fig. 2.1c). A total of 12 mortalities occurred throughout the experiment; 10 occurred among tadpoles fed the CAN diet and two in the group fed BS.



**Figure 2.1:** Comparison of mean ( $\pm$  SE) SVL (a), mass (b), and development (c) of wood frog tadpoles fed brine shrimp, fed conspecific tissues, and wild tadpoles, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for wood frog tadpoles relative to their respective experimental diets: conspecific tissues (d and e) or brine shrimp (f and g) through the duration of the 63 day study.

During the initial 14d of the experiment, tadpoles fed BS rapidly consumed dietary rations and correspondingly exhibited a mean increase in mass ( $\pm$  SE) of 0.23 ( $\pm$  0.03)g. Conversely, over the same 14d period tadpoles fed CAN did not consume their dietary rations and exhibited a mean ( $\pm$  SE) increase in mass of only 0.02 ( $\pm$  0.01)g. Multivariate analysis identified significant overall differences in growth and development between tadpoles fed BS and those fed CAN over the duration of the experiment (Day 1-63) (Hotelling-Lawley Trace:  $F_{[3,75]} = 30.2$ ,  $P < 0.001$ ). Univariate  $F$ -tests indicate that tadpoles fed the BS diet had significantly larger SVL ( $F_{[1,77]} = 35.5$ ,  $P < 0.001$ ; Fig. 2.1a), larger mass ( $F_{[1,77]} = 33.9$ ,  $P < 0.001$ ; Fig. 2.1b), and obtained significantly greater developmental stages ( $F_{[1,77]} = 38.1$ ,  $P < 0.001$ ; Fig. 2.1c) relative to tadpoles fed the CAN diet. However, tadpoles fed CAN appear to have exhibited “catch-up growth” as described by Audo et al. (1995), whereby tadpoles achieve similar mass at metamorphosis as controls but do so at the cost of prolonging their developmental period. When differences in tadpole development were accounted for between experimental treatments by testing tadpoles of overlapping developmental stages (27-32) multivariate analysis still identified overall significant differences in growth (Hotelling-Lawley Trace:  $F_{[2,39]} = 18.7$ ,  $P < 0.001$ ); univariate  $F$ -tests identified that tadpoles fed CAN were still significantly smaller in SVL ( $F_{[1,40]} = 35.8$ ,  $P < 0.001$ ) relative to tadpoles fed BS, however the two groups were not significantly different in mass ( $F_{[1,40]} = 0.4$ ,  $P = 0.5$ ) (Fig. 2.2).



**Figure 2.2:** Comparison of mean ( $\pm$  SE) snout-vent length (SVL) (**a**) and mass (**b**) of wood frog tadpoles fed conspecific tissues or brine shrimp at similar developmental (Gosner) stages.

Tadpoles fed BS exhibited similar growth and development to tadpoles from natural communities, while those fed the CAN diet exhibited inferior growth and developmental progress relative to both groups. Overall differences in SVL, mass, and development were observed between wild tadpoles and experimental (BS fed, and CAN fed) tadpoles across days 21–42 (developmental; SVL:  $F_{[2,55]} = 7.8$ ,  $P = 0.001$ ; mass:  $F_{[2,57]} = 8.7$ ,  $P = 0.001$ ; stages:  $F_{[2,57]} = 8.6$ ,  $P = 0.001$ ). Wild tadpoles had achieved significantly greater SVL ( $P <$



0.005), greater mass ( $P < 0.005$ ), and advanced developmental stages ( $P = 0.01$ ) relative to tadpoles fed CAN. There was no significant differences observed in SVL ( $P = 0.6$ ), mass ( $P = 0.3$ ), or developmental progress ( $P = 0.1$ ) between wild tadpoles and tadpoles fed BS (Fig. 2.1a,b,c).

Tadpole stable isotopic values corresponded with differences in tadpole growth between treatments. Significant differences in overall isotopic values of tadpole tissues relative to those of the diet were observed ( $F_{[1,34]} = 131.8$ ;  $P < 0.001$ ). Tadpoles fed conspecifics between days 0 and 14 were significantly enriched compared to tadpoles fed BS over the same time period ( $P = 0.001$ ), and those of tadpoles fed CAN between days 28 and 63 ( $P < 0.05$ ). Differences in isotopic values between tadpoles fed BS between days 0 and 14 and tadpoles fed conspecifics between days 28 and 63 were not significantly different ( $P = 0.6$ ). Differences in mean ( $\pm$  SE)  $\delta^{13}\text{C}$  between tadpole tissues and the CAN diet at days 0 and 14 ( $1.9 \pm 0.3\text{‰}$ ) was significantly greater than the expected value of  $1.0\text{‰}$  ( $t_{[8]} = 2.9$ ;  $P < 0.01$ ). The difference in  $\delta^{13}\text{C}$  values of of tadpole tissues and the BS diet between days 0 and 14 ( $1.1 \pm 0.2\text{‰}$ ) and conspecific fed tadpoles between days 28 and 63 ( $0.8 \pm 0.1\text{‰}$ ) were similar to the expected  $1.0\text{‰}$ . (all  $P > 0.05$ ).  $\delta^{15}\text{N}$  values of tadpoles fed CAN did not increase between days 0 and 14, while tadpoles fed BS exhibited a rapid increase in  $\delta^{15}\text{N}$  over the same time period.

Initial (day zero) mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of tadpoles were  $-27.3\text{‰}$  and  $7.3\text{‰}$ , respectively. Homogenized tadpole tissues, used to feed the CAN diet group, shifted from initial mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from  $-27.1\text{‰}$  and  $7.5\text{‰}$ , to final mean values of  $-30.4\text{‰}$  and  $4.2\text{‰}$ , respectively (Fig. 2.1d,e). This shift was due to natural ontogenetic shifts in isotopic values of tadpoles resulting from the transition to free feeding (Jefferson and Russell, 2008; Trakimas et al., 2011). Tadpoles fed CAN exhibit mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $-25.2\text{‰}$  and  $7.8\text{‰}$  at day 7, and final values of  $-29.1\text{‰}$  and  $7.1\text{‰}$  at day 63. Mean trophic

discrimination values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between days 21–63 were calculated to be approximately 1.2‰ and 2.6‰, respectively (Fig. 2.1d,e). Commercial BS offered at day 0 was slightly depleted in  $^{13}\text{C}$  and was greatly enriched in  $^{15}\text{N}$  relative to the initial values of the tadpoles (mean  $\delta^{13}\text{C}$ : -28.3‰; mean  $\delta^{15}\text{N}$ : 16.2‰).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of tadpoles fed BS rapidly increased towards equilibration with the experimental diet, achieving values of -27.2‰ and 17.2‰ (respectively) within 21 days (Fig. 2.1f,g). A change to a new batch of commercial BS prior to day 28 resulted in an unexpected shift in  $\delta^{13}\text{C}$  values. The new batch of BS exhibited a  $\delta^{13}\text{C}$  value of -21.0‰, and resulted in a rapid shift in tadpoles to a mean  $\delta^{13}\text{C}$  value of -22.0‰ by day 63 (Fig. 2.1f).  $\delta^{15}\text{N}$  values of BS also increased to 17.0‰ prior to day 28, which resulted in  $^{15}\text{N}$  enrichment of tadpoles to a final mean  $\delta^{15}\text{N}$  value of 18.9‰ at day 63 (Fig. 2.1g).

## 2.4.2 Experiment 2

Tadpoles in both the starved and BS dietary treatments exhibited the relatively high mean ( $\pm$  SE) mortality of 42.5 ( $\pm$  13.7)%; those in the BS/CM dietary treatment had a slightly lower mean ( $\pm$  SE) mortality of 35.0 ( $\pm$  8.9)%. Tadpoles in the CAN treatment exhibited mean ( $\pm$  SE) mortality of 18.8 ( $\pm$  6.2)% and those fed the CM diet had a mean ( $\pm$  SE) mortality of 11.2 ( $\pm$  2.9)%. Despite the relatively large difference in mean mortality among treatments no statistically significant differences were observed ( $F_{[4,35]} = 2.2$ ,  $P = 0.08$ ).

Tadpoles in all dietary treatments were observed foraging on the first day of feeding. However, I observed significant differences in final snout-vent length (SVL) ( $F_{[4,29]} = 36.6$ ,  $P < 0.001$ ), development ( $F_{[4,27]} = 88.4$ ,  $P < 0.001$ ), and whole body corticosterone ( $F_{[4,20]} = 15.2$ ,  $P < 0.001$ ) among treatments.

No significant differences were observed in SVL between tadpoles fed the BS and the BS/CM diets ( $P = 0.3$ ). Tadpoles fed the BS/CM diet were not significantly larger than those

fed the CAN diet ( $P = 0.08$ ), but were significantly larger than those fed the CM diet ( $P < 0.001$ ), and those in the ST treatment group ( $P < 0.001$ ). Tadpoles fed the BS diet exhibited significantly larger SVL relative to tadpoles in all other treatments (all  $P < 0.001$ ). BS fed tadpoles exhibited a mean ( $\pm$  SE) SVL of  $7.4 (\pm 0.2)$  mm, while the mean ( $\pm$  SE) mass of BS/CM fed tadpoles was  $7.1 (\pm 0.2)$  mm. No significant difference was observed in mean ( $\pm$  SE) SVL of tadpoles between the CAN ( $6.2 \pm 0.1$ mm) and CM ( $5.7 \pm 0.1$ mm) dietary treatments ( $P = 0.9$ ), however, tadpoles in both treatments were significantly larger than ST tadpoles (both  $P = 0.01$ ). Starved tadpoles were significantly smaller than tadpoles in all other treatments (all  $P < 0.01$ ) exhibiting a mean ( $\pm$  SE) SVL of  $4.5 (\pm 0.1)$  (Fig. 2.3a).

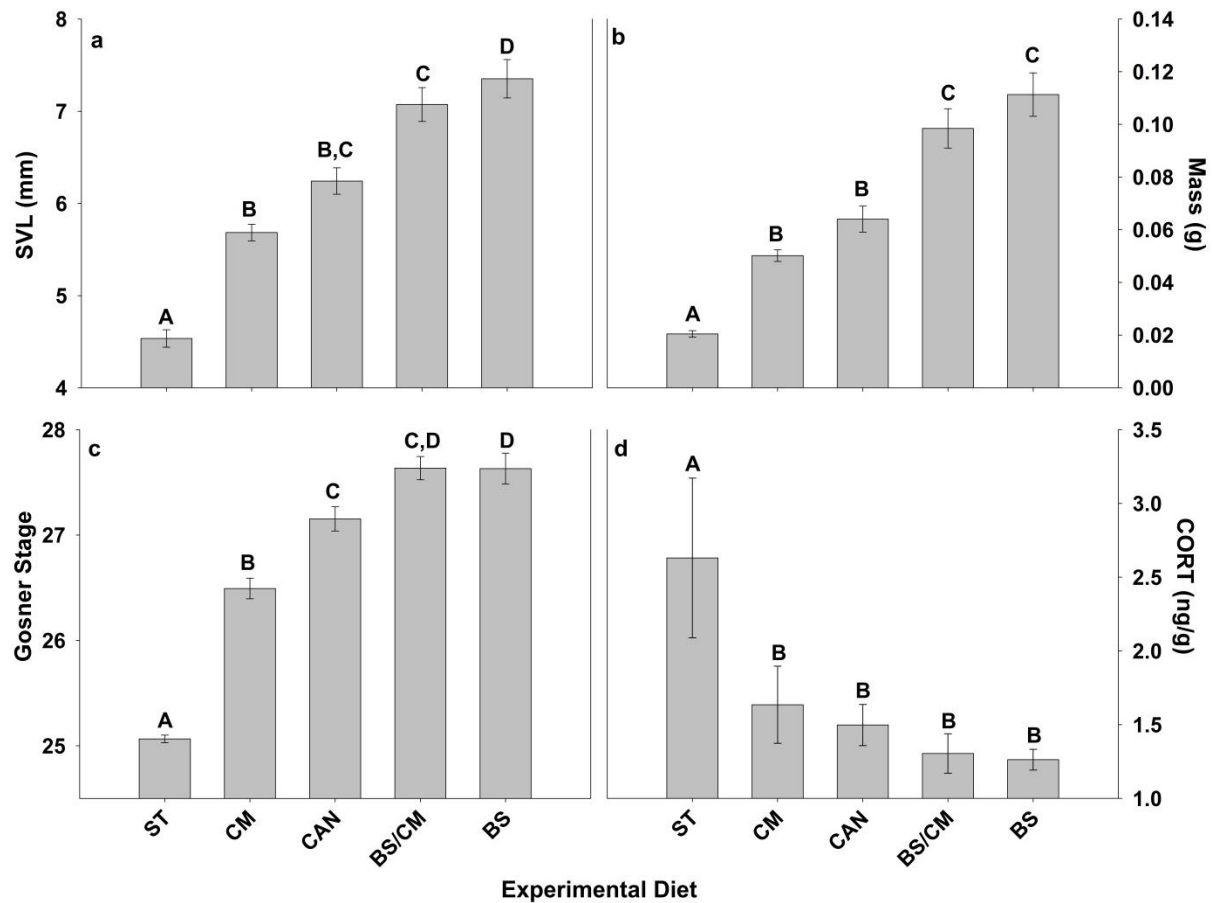
No significant differences were observed in mass between tadpoles fed the BS and the BS/CM diets ( $P = 0.6$ ), however, tadpoles in both treatments exhibited significantly higher masses relative to tadpoles in all other treatments (all  $P < 0.001$ ). HP fed tadpoles exhibited a mean ( $\pm$  SE) mass of  $0.11 \text{ g} (\pm 0.01)$ , while the mean ( $\pm$  SE) mass of BS/CM fed tadpoles was  $0.10 \text{ g} (\pm 0.01)$ . No significant difference was observed in mean ( $\pm$  SE) masses of tadpoles between the CAN ( $0.06 \pm 0.01 \text{ g}$ ) and CM ( $0.05 \pm 0.00 \text{ g}$ ) dietary treatments ( $P = 0.9$ ), however, tadpoles in both treatments were significantly larger than ST tadpoles (CAN – ST:  $P < 0.001$ ; LP – ST:  $P = 0.01$ ). Starved tadpoles were significantly smaller than tadpoles in all other treatments (all  $P < 0.01$ ) exhibiting a mean ( $\pm$  SE) mass of  $0.02 \text{ g} (\pm 0.00)$  (Fig. 2.3b).

Starved tadpoles exhibited a significantly lower mean ( $\pm$  SE) final developmental stage ( $25.1 \pm 0.0$ ) than tadpoles in all other treatments (all  $P < 0.001$ ). Tadpoles fed the CM diet exhibited a mean ( $\pm$  SE) final developmental stage of  $26.5 (\pm 0.1)$ , which was significantly lower than tadpoles fed the BS diet ( $P < 0.001$ ), the BS/CM diet ( $P < 0.001$ ), and those fed the CAN diet ( $P < 0.005$ ). Tadpoles fed the BS/CM diet had a mean ( $\pm$  SE) final developmental stage of  $27.7 (\pm 0.1)$ , which was not significantly different from those

fed the CAN diet ( $27.2 \pm 0.1$ ;  $P = 0.18$ ), or those fed the BS diet ( $27.7 \pm 0.2$ ;  $P = 0.62$ ).

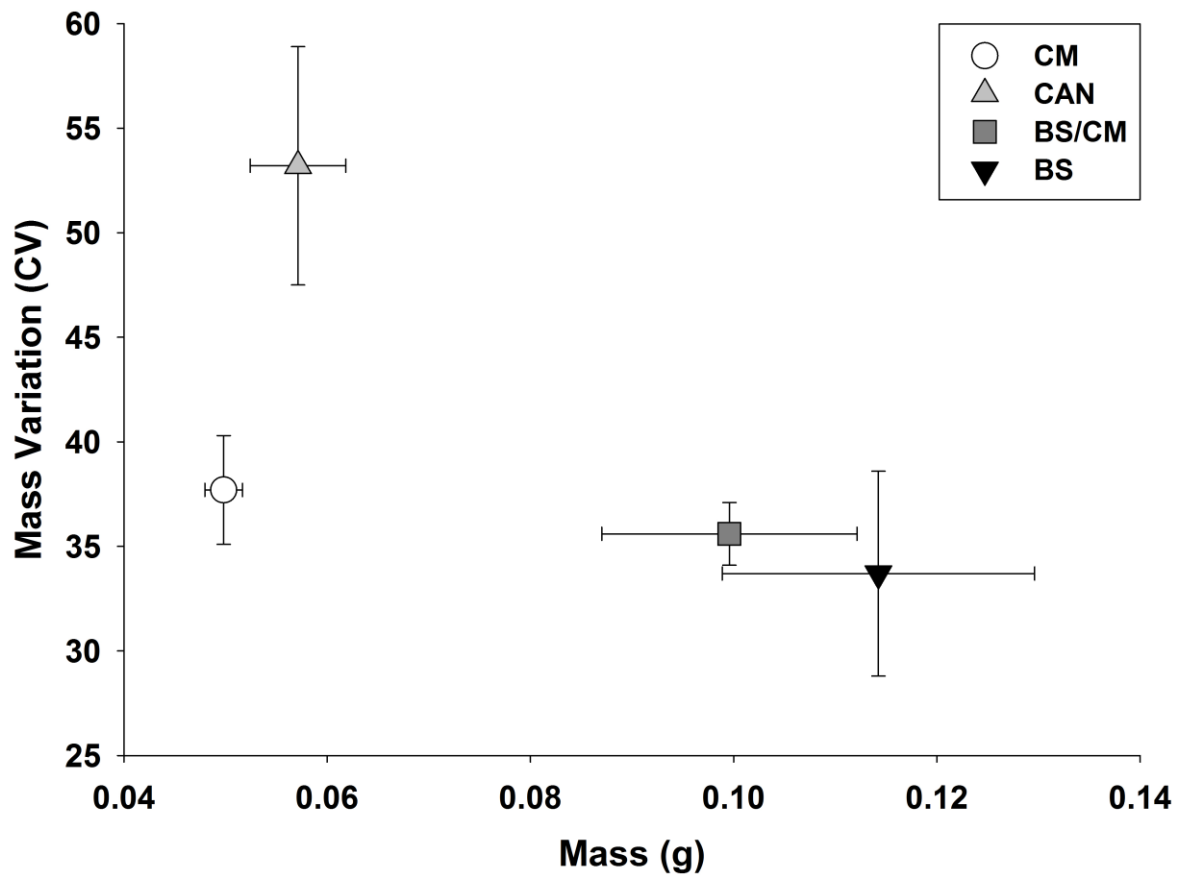
However, tadpoles fed the BS diet had a significantly higher final developmental stage than tadpoles fed the CAN diet ( $P = 0.01$ ) (Fig. 2.3c).

Starved tadpoles exhibited a significantly higher mean ( $\pm$  SE) corticosterone level ( $2.63 \pm 0.54\text{ng}\cdot\text{g}^{-1}$ ) than tadpoles in all other treatments (all  $P < 0.001$ ). However, no other significant differences were observed in mean ( $\pm$  SE) corticosterone levels (BS:  $1.26 \pm 0.07\text{ng}\cdot\text{g}^{-1}$ ; CM:  $1.64 \pm 0.26\text{ng}\cdot\text{g}^{-1}$ ; BS/CM:  $1.30 \pm 0.13\text{ng}\cdot\text{g}^{-1}$ ; CAN:  $1.49 \pm 0.14\text{ng}\cdot\text{g}^{-1}$ ) among treatments (all  $P > 0.10$ ) (Fig. 2.3d).



**Figure 2.3:** Comparison of relative growth, development, and hormonal response of tadpoles among experimental diets. Mean ( $\pm$  SE) values of final wood frog tadpole snout-vent length (a), mass (b) final developmental stage (c), and whole body corticosterone (d) for each experimental diet in experiment two: starvation (ST), low protein cornmeal diet (CM), cannibalistic/conspecific tissues diet (CAN), mixed (50% BS and 50% CM) diet BS/CM, and high protein brine shrimp diet (BS).

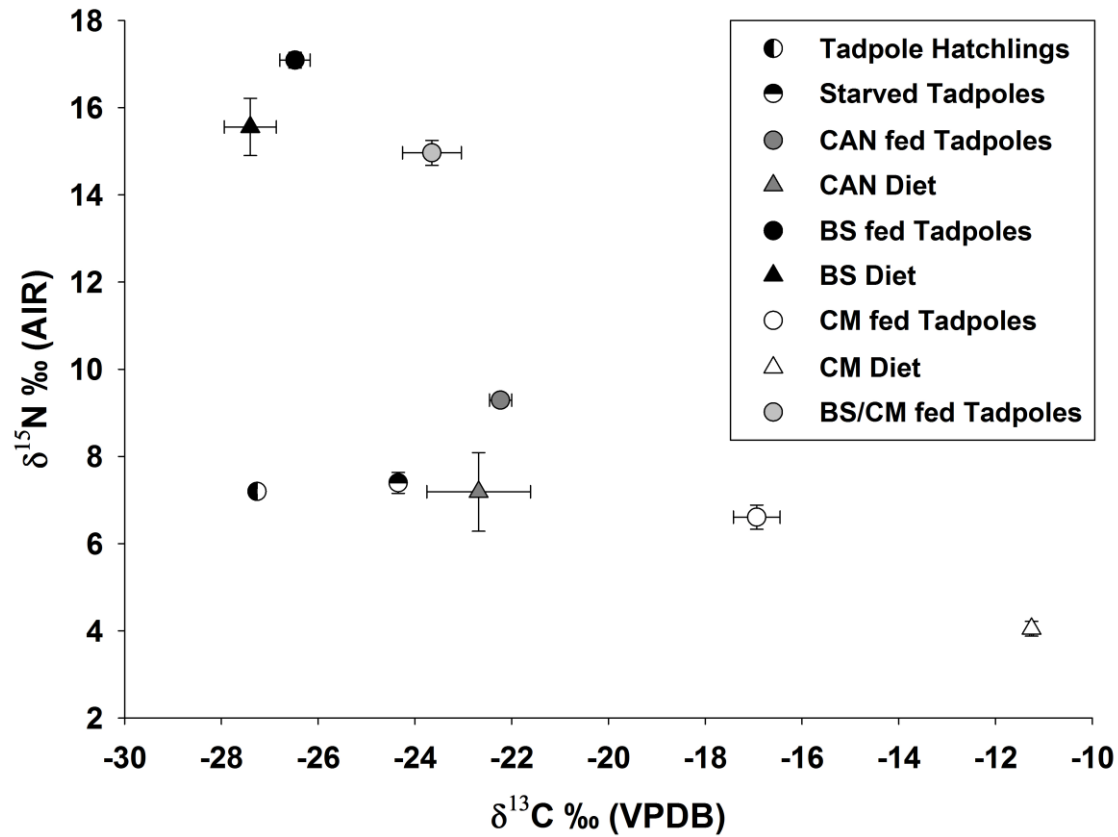
Analysis of intraspecific variation in SVL of tadpoles indicated overall differences among treatments ( $F_{[3,22]} = 10.9$ ,  $P < 0.001$ ). Interestingly, tadpoles in the CAN treatment exhibited the highest %CV at  $53.2 \pm 5.7\%$  (mean  $\pm$  SE), relative to all other treatments (all  $P < 0.005$ ). Pairwise comparisons among all other treatments were not significantly different (all  $P > 0.5$ ); whereby tadpoles exhibited a mean ( $\pm$  SE) %CV of  $33.7 \pm 4.9\%$  in the BS treatment,  $37.7 \pm 2.6\%$  in the CM treatment, and  $35.6 \pm 1.5\%$  in the BS/CM treatment. This indicates a relatively greater disparity in size among individual tadpoles fed the CAN diet than tadpoles fed any other diet (Fig. 2.4).



**Figure 2.4:** Relative variation of mass, mean ( $\pm$  SE) CV, of tadpoles as a function of their mean ( $\pm$  SE) mass for each of the experimental diets in experiment two, except the starved group which was excluded: low protein cornmeal diet (CM), cannibalistic diet (CAN), mixed (50% BS and 50% CM) diet (BS/CM), and high protein brine shrimp diet (BS). Values were calculated as means of individuals within each aquarium, which were subsequently used to produce mean ( $\pm$  SE) values among aquaria for each treatment.

Starved tadpoles exhibited a similar shift from initial stable isotopic values as observed in cannibalistic tadpoles at day 14 in Experiment 1. Mean ( $\pm$  SE)  $\delta^{13}\text{C}$  values of starved tadpoles ( $-24.3 \pm 0.1\text{‰}$ ) were significantly higher than those of initial tadpole values ( $-27.3 \pm 0.0\text{‰}$ ;  $t_{[1.6]} = 15.3$ ,  $P = 0.01$ ). Conversely  $\delta^{15}\text{N}$  values were not different between starved tadpoles ( $7.4 \pm 0.2\text{‰}$ ) and initial values ( $7.2 \pm 0.1\text{‰}$ ;  $t_{[5.7]} = 5.7$ ,  $P = 1.0$ ; Fig. 2.5). This result appears consistent with that of tadpoles fed CAN within the initial 14d of

Experiment 1, confirming these tadpoles experienced a period of starvation during this time period.



**Figure 2.5:** Comparison of tadpoles relative to their respective diets and initial wild tadpole values. Mean ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of wood frog tadpoles in each of the feeding treatments in experiment two. Tadpoles in each experimental diet group are denoted using the following abbreviations: starvation (ST), low protein cornmeal diet (CM), cannibalistic diet (CAN), mixed (50% BS and 50% CM) diet (BS/CM), and high protein brine shrimp diet (BS). Experimental diets are denoted by their full description.

Tadpoles provided the mixed diet appeared to preferentially consume BS over CM. Proportional assimilation estimates produced using SIAR indicate that the mean ( $\pm$  SE) contribution of BS to the assimilated diet was 74 ( $\pm$  3.5) %, while that of CM was 26 ( $\pm$  3.4) %. This is consistent with personal, though un-quantified observations of tadpole feeding; BS was sought after and rapidly consumed, while CM appeared to be a collateral resource,

inadvertently consumed in the process of feeding on BS. In the absence of BS, tadpoles exhibited relatively passive foraging behaviours, lacking the same attraction to food as they exhibit towards BS. In all experimental aquaria (including stock aquaria used to raise tadpoles to produce the cannibal diet) where tadpoles competed for BS, aggressive behaviour (i.e. biting) was observed among tadpoles as they vigorously competed for this resource. Occasionally, large tadpoles were observed cannibalizing smaller live conspecifics as a result of such encounters. This may then partially account for the relatively high mortality in the BS and BS/CM treatments.

### **2.4.3 Experiment 3**

Tadpoles in stock aquaria were casually observed to form large feeding aggregations around patches of food resources, often within 30s of either diet being offered. Aggression was commonly observed among competing individuals, which was on multiple occasions observed leading further on to cannibalistic behaviour. Cannibalism was initially observed in stock aquaria within 8d of hatching, and within 2d of initiating free-feeding, at stage 25 of development. The initial observation of cannibalistic behaviour involved a relatively larger individual attacking a smaller individual from above and behind. The victim was attacked, while feeding, from an aggressor using an attack similar to that described by Petranksa and Thomas (1995). The attacker assumed a head-down position aimed at the victim and repeatedly bit near the dorsal posterior end of the victim's body, propelling itself at the victim with thrusts from its tail (Petranksa and Thomas 1995). Similar observations were described by Caldwell and Araújo (1998) in interspecific predation between *Dendrobates auratus* (prey) and *Rana warschewitschii* (predator), and between *D. castaneoticus* (predator) and *Epipedeobates femoralis* (prey). Additional tadpoles rapidly began joining the original aggressor in frenzied feeding upon the dead or dying victim, similar to the situations

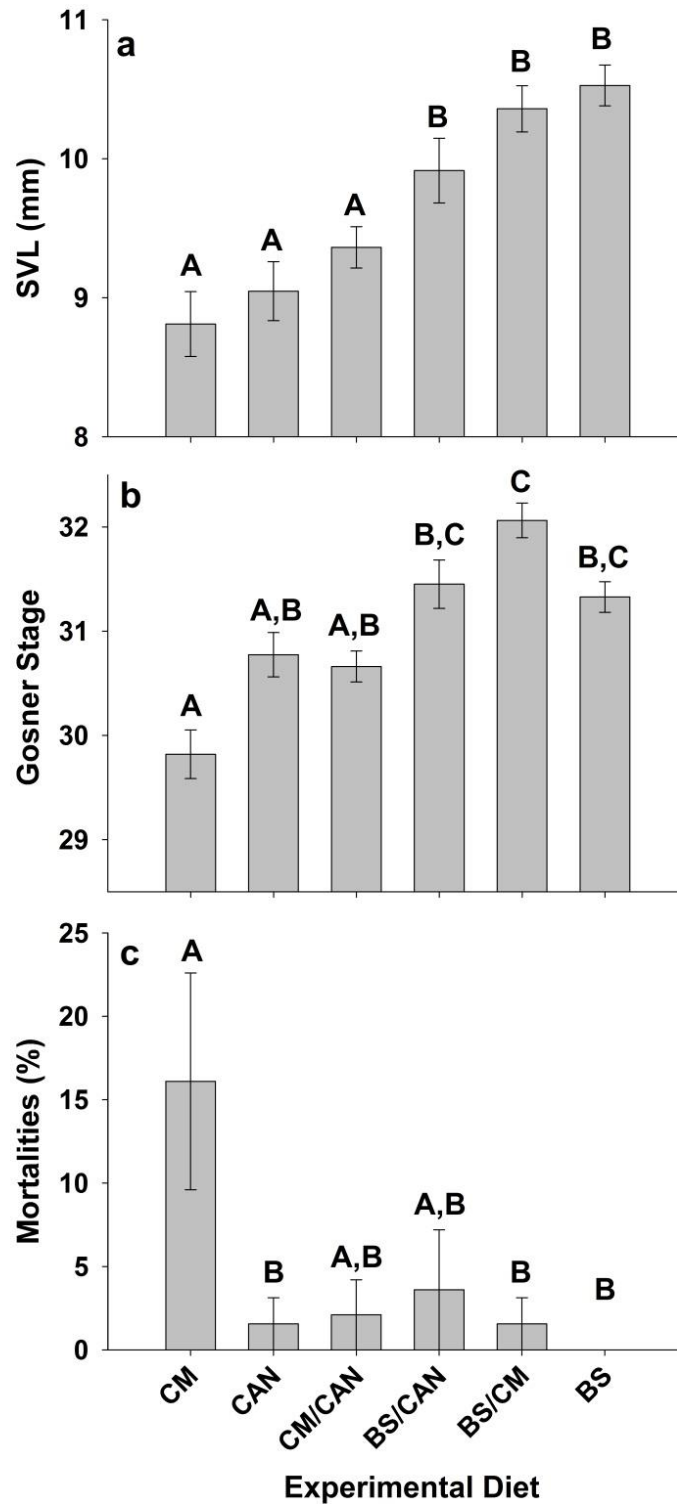


described by Petranksa and Thomas (1995), and Crump (1986). In the feeding melee up to five or six individuals were observed swarming the victim until the body was consumed. Similar situations were observed at multiple times throughout the study.

Tadpoles in all dietary treatments were observed foraging on experimental diets from the initial day of experimentation. However, tadpoles exhibited overall significant differences in final snout-vent lengths (SVL) among dietary treatments ( $F_{[5,41]} = 13.3$ ,  $P < 0.001$ ). A clear divide was observed in SVL between those tadpoles fed a diet containing BS and those fed diets that lacked BS. Mean ( $\pm$  SE) SVL of tadpoles fed a diet with BS exhibited no significant difference among each other (BS:  $10.6 \pm 0.1$ mm; BS/CAN:  $9.9 \pm 0.2$ mm; BS/CM:  $10.4 \pm 0.2$ mm; all  $P > 0.2$ ), but were all significantly larger than those fed diets without BS (all  $P \leq 0.02$ ). Similarly, all tadpoles fed diets lacking BS exhibited no significant differences in mean ( $\pm$  SE) SVL (CM:  $8.8 \pm 0.2$ mm; CAN:  $9.0 \pm 0.2$ mm; CM/CAN:  $9.4 \pm 0.1$  mm) (all  $P > 0.3$ ; Fig. 2.6a).

Overall, significant differences were observed in the developmental progress of tadpoles among dietary treatments ( $F_{[5,41]} = 10.9$ ,  $P < 0.001$ ). Differences in final developmental stages in tadpoles among dietary treatments were less straight-forward than those observed for SVL values. Tadpoles fed CM exhibited the lowest mean ( $\pm$  SE) final developmental stage ( $29.8 \pm 0.3$ ), which was significantly lower than tadpoles fed BS/CAN ( $31.4 \pm 0.2$ ;  $P < 0.001$ ), BS/CM ( $32.1 \pm 0.2$ ;  $P < 0.001$ ), and BS ( $31.3 \pm 0.2$ ;  $P = 0.001$ ), but were not significantly different from tadpoles fed CAN ( $30.8 \pm 0.2$ ;  $P = 0.07$ ) or CM/CAN ( $30.7 \pm 0.2$ ;  $P = 0.1$ ). Similarly, no significant differences were observed in final developmental stages among tadpoles fed CAN, CM/CAN, BS/CAN, and BS (all  $P > 0.1$ ), and no significant differences were observed among tadpoles fed BS/CAN, BS/CM, and BS (all  $P > 0.1$ ), however, tadpoles fed BS/CM exhibited a significantly higher final developmental stage than tadpoles fed CAN ( $P < 0.005$ ) and CM/CAN ( $P = 0.001$ ; Fig. 2.6b).

Unlike observations from Experiment 2, tadpoles fed at later stages differed in overall percent mortality among dietary treatments ( $F_{[5,38]} = 3.5$ ;  $P = 0.01$ ). Tadpoles fed CM had the highest mean ( $\pm$  SE) percent mortality among all treatment groups ( $16.1 \pm 6.5\%$ ), which was significantly greater than the mortality observed for tadpoles fed diets of CAN ( $1.6 \pm 1.6\%$ ;  $P < 0.05$ ), BS/CM ( $1.6 \pm 1.6\%$ ;  $P < 0.05$ ), and BS ( $0 \pm 0\%$ ;  $P < 0.01$ ). No significant difference in mean ( $\pm$  SE) percent mortality was observed between BS/CAN ( $3.6 \pm 3.6\%$ ) and CM/CAN ( $2.1 \pm 2.1\%$ ;  $P = 1.0$ ), nor were tadpoles from these treatments observed to differ from those of any other treatment (all  $P > 0.05$ ; Fig. 2.6c).



**Figure 2.6:** Comparison of growth, development, and percent mortality of tadpoles among experimental diets. Mean ( $\pm$  SE) values of snout-vent length (a), final developmental stage (b), and percent mortality (c) of tadpoles in each feeding treatment in experiment three: low protein cornmeal diet (CM), high protein brine shrimp diet (BS), cannibalistic diet (CAN), mixed 50% CM and 50% CAN diet (CM/CAN), mixed 50% BS and 50% CAN diet (BS/CAN), and mixed 50% BS and 50% CM diet (BS/CM).

## 2.5 Discussion

Cannibalism can be a highly profitable strategy, yielding a theoretically high quality diet by composition while diminishing intraspecific competition (Fox 1975; Meffe and Crump 1987). Wood frog tadpoles are capable predators and efficient cannibals upon embryonic and newly hatched larval amphibians (Holbrook et al. 2004; Petranka and Thomas 1995; Petranka et al. 1998). Therefore, a diet consisting of conspecific tissues should be highly beneficial to tadpole growth and development. However, the results of my experiments here indicate tadpoles experience relatively poor growth and development when provided an exclusively cannibalistic diet.

Individually housed tadpoles provided the CAN diet starved for the initial 14d feeding period. This is supported by the results of stable isotope analyses and comparison of results from the starvation treatment in Experiment 2. Lack of  $^{15}\text{N}$  enrichment suggests cannibal tadpoles did not metabolize protein during this period (Gannes et al. 1997; McCue 2007). Therefore, the increase in whole body  $\delta^{13}\text{C}$  values of cannibalistic tadpoles suggests they metabolized only stored lipids. Lipid metabolism resulting from starvation would result in rapid loss of  $^{13}\text{C}$  depleted lipids leading to an increase in total body  $\delta^{13}\text{C}$  values, while leaving  $\delta^{15}\text{N}$  values relatively unchanged. The subsequent shift in isotopic values between tadpole tissues and the CAN diet that appears from day 28 to day 60 corresponds with the expected values of free feeding tadpoles ( $\sim 1.0\text{‰}$ ).

Tadpoles raised in groups fed immediately and successfully upon CAN; therefore it does not appear that the individually-housed tadpoles starved as a result of an inability to locate, consume, or assimilate CAN. Individually-housed tadpoles appear to have preferentially avoided consuming CAN during the initial 14d period, suggesting that tadpoles responded to a risk cue emanating from the homogenized tissues. Kinship cues are unlikely to initiate this response because they were composed of individuals from multiple egg clutches

and from multiple ponds. Similarly, prior experiments suggest that tadpoles maintain feeding under risk of predation, and that hunger results in increased feeding regardless of apparent risk (Bridges 2002; Horat and Semlitsch 1994). Similarly, in a prior experiment I observed that chemical cues of homogenized conspecifics alone did not dissuade tadpoles from feeding, but may have instead been interpreted as competitive cues resulting in larger tadpole bodies [Jefferson et al., 2013(Chapter 3)]. However, Jefferson et al. (2013; Chapter 3) combined cues from macerated conspecifics with a profitable diet; this may have initiated a conflict in information between putative alarm cues and profitable dietary cues. In my current study the source of the putative alarm cues was also the diet itself, providing no conflicting cue to potentially lure the tadpoles in to feed. Jefferson et al. (2014; Chapter 4) seems to support this, showing that tadpoles responded to conspecific tissues more rapidly when combined with the chemical cues of an alternative diet.

Tadpoles may have also avoided feeding on CAN due to potential for acquisition of pathogens (Pfennig et al., 1998). Kiesecker et al. (1999) observed that bullfrog tadpoles preferentially avoid conspecifics infected with pathogenic yeast (*Candida humicola*) as identified by chemical cues. I initially attempted to limit such risk by collecting pre-free-feeding tadpoles. Additionally, tadpoles raised in groups began feeding immediately. This may have been the result of differences in pathogen exposure of tadpoles between experiments. Conversely, the difference in feeding response may simply have been a result of tadpole exposure to actual competition. Relyea (2002) observed that wood frog tadpoles exhibit increasing activity with intraspecific competition. Therefore, competitive effects may have preceded those of putative risk cues. This is also supported by Jefferson et al. (2014; Chapter 4) who observed that tadpoles provided a diet of conspecific tissues would initiate feeding more rapidly in the presence of a competitor than when fed individually.

Audo et al. (1995) found that tadpoles starved in early development experienced prolonged developmental periods. The duration of the nutritional stress experienced by individual cannibalistic tadpoles and their developmental stage at which they experienced nutritional stress were similar to those of tadpoles in the early starvation treatment procedure in Auto et al. (1995). These cannibalistic tadpoles, having experienced approximately 14d of starvation, similarly exhibited the prolonged larval developmental and apparent “catch-up growth” (Fig. 2.2) observed by Auto et al. (1995).

McCallum and Trauth (2002) observed that tadpoles fed a 100% CM diet were smaller, required longer time to metamorphosis, and had lower survival than tadpoles fed diets with a greater proportion of protein (100% soybean). Babbitt and Meshaka (2000) suggest that cannibalism among free-feeding tadpoles is an adaptation to low food quality and/or food abundance rather than one of pond drying, due to the observation of prolonged larval development among tadpoles fed a high-quality diet and conspecifics tissues. Interestingly, results of Experiment 2 suggest that a cannibalistic diet facilitated a developmental rate that was greater than that of tadpoles fed the low quality CM diet and similar to that of tadpoles fed the mixed diet. However, despite the deficits of CM as a diet, my results indicate that short-term obligatory feeding upon this low quality diet failed to elicit the elevated corticosterone levels suggested by Venesky et al. (2012). Therefore, these conditions may be insufficient to elicit the same feeding response upon CAN exhibited by starved tadpoles. It may be that long-term obligatory feeding on such a diet may eventually stimulate elevated corticosterone levels and elicit such a response, however, this appears to require longer than the 14d feeding period tested. Since my results also indicate no difference in growth of cannibalistic tadpoles to those fed CM, but significantly lower than tadpoles fed the mixed or high quality BS diets, it does not appear that cannibalism is simply an adaptive response to low dietary resource quality.

Interestingly, the results of the late stage feeding experiment suggest that tadpoles initially raised on a mixed diet of BS/CM did not appear to exhibit any improvement in their response to a cannibalistic diet, relative to tadpoles fed a cannibalistic diet from the initiation of free feeding. Additionally, Babbitt and Meshaka (2000) found that the addition of conspecific tissues as a supplementary diet improved growth and development of tadpoles where the base diet was of low quality (algae), but was detrimental to those fed a high quality basal diet (rabbit chow). However, in my late stage feeding experiment I failed to observe this detrimental effect. I found that tadpoles responded no differently to the combination of conspecific tissues with a high quality (BS) diet in either growth or development relative to tadpoles fed diets of BS, or mixed BS/CM diets. However, when tadpoles were fed a diet of conspecific tissues with the low quality CM diet they exhibited slight improvement in development relative to tadpoles fed the CM diet alone, but were no different than tadpoles fed the CAN diet. Tadpoles fed the CAN diet in the late stage feeding experiment appear not to have benefited from this diet to the same extent as those in the early stage feeding experiment had in terms of SVL growth and development (compare Fig. 2.3a,c and Fig. 2.6a,b). It is possible that tadpoles had become conditioned to their initial control diet of BS/CM and required a transition period to the experimental diets. This may be supported by the relatively high mortality of tadpoles fed CM alone; having been previously provided a diet with both CM and BS tadpoles may have been unable to cope with the rapid decline in dietary quality. The relatively low protein content or possibly the indigestibility of CM may have been a major factor since mortalities in all other dietary treatments were relatively low. It is also possible that the observational period for this experiment was too short and that differences between groups would have become increasingly apparent over time.

Tadpoles have traditionally been considered as indiscriminate foragers (Dickman 1968), suspension-feeders, herbivores, and detritivores (Duellman and Trueb 1986; Altig et al.

2007). However, when I presented tadpoles with the 50:50 mix of high and low protein diets, they assimilated a disproportionately high amount of BS relative to CM (Fig. 2.5). Secco et al. (2005) suggest CM represents a relatively digestible diet for bullfrog tadpoles (*Lithobates catesbeianus*), however, my results suggest it may be less so for wood frog tadpoles. Additionally, CM was always present, even after BS was depleted, and tadpoles were rarely observed actively consuming CM. It is possible that tadpoles selectively consumed BS over CM to ideally facilitate growth and/or development as suggested by Richter-Boix et al. (2007). Tadpoles were observed excreting cornmeal that appeared undigested, in both Experiments 2 and 3. This further suggests that wood frog tadpoles were unable to properly digest CM and may have required coprophagy to extract sufficient energy and nutrient from this diet.

Alternatively, tadpoles may also have responded to attractive chemical cues released from BS, while ignoring CM or CAN diets due to a lack of any preferential cues. Therefore, the efficacy of diets lacking attractive cues may be hindered by the inability of tadpoles to identify them as profitable resources. Heinen and Abdella (2005) observed that American toads would not cannibalize injured live conspecifics, but may consume dead conspecifics. The authors suggest that the feeding response is stimulated by chemical cues that are only released as a result of bacterial decomposition. While this is certainly possible, it does not explain why tadpoles in Experiment 1 tolerated nearly two weeks of starvation before consuming conspecific tissues, while tadpoles housed in groups began feeding immediately.

An alternative proximal trigger of cannibalistic behaviour may be the presence of a high quality diet (i.e. BS). My observations of aggressive feeding behaviour are consistent with those of Waldman (1985), who noted that when food resources are limited tadpoles will “displace conspecifics feeding on an attractive food source”. While the resources themselves were not limited (I provided the tank with sufficient food to last the 48h feeding periods)



these resources were often unevenly distributed within aquaria, allowing certain individuals to monopolize profitable feeding areas. Individual tadpoles exhibiting relatively greater competitive success may then have grown larger, and this pattern of aggressive behaviour could lead to encounters resulting in cannibalism.

Peacor and Pfister (2006) stated that differences in intra-population growth rates, and thus size variation can arise from differences in individual growth rates ('size-dependent'), and through phenotypic differences in foraging among individuals ('size-independent'). Peacor and Pfister (2006) also observed that 'size-dependent' factors appear to be responsible for differences in sizes at lower competition, while 'size-independent' factors appear primarily responsible for intra-population size variation at greater competition levels. Acquisition of limited resources can be improved in individuals with advantageous traits, and thus worsened in those without (Peacor and Pfister 2006). The effects of these traits then become increasingly pronounced with increasing competition, and thus an increased size disparity develops among individuals within the population (Peacor and Pfister 2006). I therefore expected that the greatest size discrepancy would have been observed among tadpoles in dietary treatments including BS, due to its high profitability and attractiveness. However, the observation that the greatest size-variation was among tadpoles fed CAN may still be congruent with the findings of Peacor and Pfister (2006). My initial results from Experiment 1 indicate that individual tadpoles may have an aversion to consuming CAN. However, if some individuals exhibit a phenotypic foraging trait that may encourage consumption of these tissues (e.g. boldness) then this would similarly explain the discrepancy among individuals despite the lack of apparent competition among individuals for this food resource. Additionally, the aggression observed among tadpoles provided BS in their diets may act to reduce size disparity within populations by increasing mortality of smaller individuals. Although I did not observe any such evidence for this in my study, my

observations of size dependent aggression and cannibalism among BS fed individuals suggest this is a possibility. Similarly, McCallum and Trauth (2001) observed that the Illinois chorus frog (*Pseudacris streckeri*) exhibited cannibalistic behaviour that may have contributed to mortality within their experiment.

The results of this study suggest that cannibalism is not the high quality diet it is theorized to be, but may at least facilitate survival to metamorphosis, and may yield benefits to development over low protein diets. Factors influencing the expression of this behaviour, however, are far more convoluted. My results indicate that cannibalism certainly occurs in response to extreme nutritional deficiencies, but may also quickly arise through aggressive encounters resulting from competition for profitable diets. Although aggressive behaviour among tadpoles fed low quality diets appeared to be absent in this study, this does not necessarily indicate that tadpoles will not resort to cannibalistic behaviour at a later period. Under more dire circumstances (e.g. pond drying, increased competition, increased predation, etc.) requiring increased protein in the tadpoles' diet to improve growth or development, cannibalism may fulfill such necessary protein requirements.

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## **Chapter 3: Understanding the information value of repeated exposure to chemical alarm cues: what can growth patterns tell us?**

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### **3.1 Abstract**

Chemical cues released into the environment from injured prey animals provide a rich source of information about ambient risk. However, these cues could also provide information not associated with predation risk. Here I exposed wood frog tadpoles (*Lithobates sylvaticus*) to a control diet and one that was soaked in chemical cues released from injured conspecifics and documented growth and development of the tadpoles. If animals perceive repeated exposure to injured conspecific cues as indicating a high risk environment, then I predict that tadpoles would reduce foraging, prolonging time to metamorphosis, reducing growth rate and initiating adaptive changes in tail morphology. Conversely, if tadpoles interpret repeated exposure to these chemical cues as an indicator of competitor density, they should increase growth rate and body size to become better competitors. I found that tadpoles exposed to chemical cues exhibited significantly larger body width and body length relative to the control. These patterns are inconsistent with a response to risk but correspond with observed responses of wood frog tadpoles to increased competition.

### 3.2 Introduction

The survival of organisms is dependent on their ability to detect and respond appropriately to various sources of information (Relyea 2004, Pohnert et al., 2007; Moir and Weissburg, 2009; Schoeppner and Relyea, 2009). Any given species may improve its survivorship by investing in adaptations to evade or deter predation (Dawkins and Krebs, 1979), to limit exposure to pathogens (Kiesecker et al. 1999), to survive inclement environmental conditions (Wellborn et al., 1996; Griffiths, 1997; Richter-Boix et al., 2007), and to improve competitive success (Relyea, 2002; Relyea and Auld, 2004; Michel, 2012). Various sensory cues are used to track and assess ecological conditions (Petranka and Hayes, 1998). Indeed, many species utilize chemical sources of information to identify the origin and degree of potential predation threat (reviewed by Chivers and Smith, 1998; Ferrari et al., 2010) and the level of competition (Relyea, 2002; Michel, 2012).

When prey are captured by predators, they often release chemical cues into the environment. These cues often induce avoidance behaviour in conspecifics (i.e. reduced activity, shelter seeking, and schooling) and hence are often referred to as alarm cues (Hagman and Shine, 2008; Moir and Weissburg, 2009; Schoeppner and Relyea, 2009). Alarm cues that are ingested by predators may also induce the expression of phenotypically plastic morphological traits (i.e. larger bodies, modified locomotory structures) (Van Buskirk and McCollum, 2000; Kishida and Nishimura, 2004; Relyea, 2004; Richter-Boix et al., 2007; Chivers et al., 2008). Indeed, the responses can be fine-tuned to respond to the nature and intensity of the threat, and the resource availability of the habitat (Relyea, 2004). The ability to produce context-dependent responses to specific threats can dramatically improve survivorship, while minimizing the incurred cost of the response (Van Buskirk and McCollum, 2000; Richter-Boix et al., 2007). Therefore, an evolutionary pressure may exist towards the expression of rapid and efficient responses to highly variable conditions

(Dawkins and Krebs, 1979; Van Buskirk and McCollum, 2000; Relyea and Auld, 2004; Richter-Boix et al., 2007).

An issue not typically appreciated by researchers studying alarm cues is that such cues can, in fact, provide prey with additional pieces of ecologically relevant information that are not linked to risk. For example, conspecifics may release a suite of informative cues when injured and an individual could interpret information on the frequency of attacks on conspecifics to determine the level of potential competition (Relyea, 2002). All else being equal, increasing cue concentrations in the vicinity may identify a higher density of competitors (Relyea, 2002).

Cannibalism represents a potentially profitable foraging strategy because conspecific tissues contain all essential nutrients, presumably in the appropriate proportions for growth and development (Greenstone, 1979; Meffe and Crump, 1987; Wildy et al., 1998). Such a diet can improve growth, development, and/or survivorship of individuals experiencing dietary stress resulting from reductions in quality and/or quantity of dietary resources (Fox, 1975; Meffe and Crump, 1987; Babbitt and Meshaka, 2000). Incidental exposure to alarm cues released from victims could, however, dissuade cannibalism by invoking avoidance behaviour in the attackers. Conversely, Crump (1986) observed that initiation of cannibalism among Cuban treefrog tadpoles (*Osteopilus septentrionalis*) appeared to attract additional tadpoles to attack, and this behaviour may have been stimulated by chemical cues. Similarly, Crossland and Shine (2011) identified chemical cues released from late stage embryonic cane toad tadpoles (*Bufo marinus*) that stimulate older, free-feeding conspecifics to cannibalize embryos. Heinen and Abdella (2005) observed that older tadpoles of American toads (*Anaxyrus americanus*) were quicker to consume conspecific tissues than younger individuals. Exposure to conspecific cues may therefore dissuade or promote cannibalism, depending on the ecological context.

The objective of this work was to study growth patterns of tadpoles to assess how tadpoles interpret repeated information indicating that conspecifics in the vicinity were captured by predators. Theory developed from a risk perspective predicts that tadpoles exposed to alarm cues would show immediate anti-predator behaviour (e.g. Chivers and Smith, 1998; Ferrari et al., 2008; Moir and Weissburg, 2009). Over the long term this would lead to a reduction in foraging with the consequence of increasing time to metamorphosis (Audo et al., 1995), lowering growth rate (Crespi and Denver, 2004, Schoeppner and Relyea 2005; Fraker et al., 2009; Schoeppner and Relyea, 2009) and may initiate adaptive changes in tail morphology (Schoeppner and Relyea, 2005). In contrast, if tadpoles interpret repeated exposure to alarm cues as an indicator of competitor density, they should have a different pattern of growth (Relyea, 2002; Relyea, 2004). Specifically they should increase growth rate and body size (width and length) to become better competitors (Relyea, 2002; Michel, 2012).

### **3.3 Materials and Methods**

#### ***3.3.1 Specimen collection***

I collected wood frog egg masses on May 3, 2011 from multiple populations in disjunct wetlands in the Nisbet Provincial Forest area north of Prince Albert, SK, Canada (N 53°17.188', W 105°39.226'). All wetlands were primarily open canopy; however each differed in its proximity to forest cover. All wetlands contained semi-aquatic or biphasic species of predatory invertebrates (e.g. odonates and dytiscids), while only the more permanent wetlands contained species incapable of evading drying periods (i.e. leeches). Plains garter snakes (*Thamnophis radix*) are common and ubiquitous throughout the area and may pose a threat to tadpoles in all collection sites, as do many predatory birds and mammals (e.g. striped skunks: *Mephitis mephitis*; North American raccoons: *Procyon lotor*; red foxes:

*Vulpes vulpes*; coyotes: *Canis latrans*; etc). Adult barred tiger salamanders (*Ambystoma mavortium*) are also commonly observed in the area. Egg masses were transported to the University of Saskatchewan, and maintained in a 38 L glass aquarium filled with room temperature de-chlorinated water and pond water.

### **3.3.2 Experimental Procedures**

A total of 90 tadpoles at Gosner (1960) stage 25 were individually housed in 980 mL (136.7 x 136.7 x 69.9 mm) plastic containers and randomly assigned to 1 of 2 treatments (45 tadpoles per treatment) in a randomized block arrangement. Tadpoles received water changes 3 days a week (M, W, F) and were fed immediately after all water changes were completed. Water quality (i.e. pH, NH<sub>3</sub>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, GH, KH, Cl) of experimental containers was assessed a minimum of once a week within 24 hours of a feeding using commercially available aquarium test strips. Experiments were performed under a controlled 14:10 h (light:dark) photoperiod. Mean ( $\pm$  SE) water temperatures for experimental containers were 19.7 °C ( $\pm$  0.1) through the course of the experiment.

Tadpoles were fed a diet of frozen brine shrimp (72.5% dry protein) and generic rodent pellet food (18% dry protein) consistently mixed to a 4:1 ratio by mass. This combined diet was prepared to provide a relatively high protein diet (Schiesari et al., 2009) while providing high resource dispersal to encourage constant foraging and minimize water fouling. Tadpole food was subsequently placed in a solution of conspecific cues in de-chlorinated water (exposure group) or de-chlorinated water alone (control group), homogenized, and allowed to soak in solution for a minimum of 30 min prior to feeding. Conspecific cues were produced by macerating 0.5 g of tadpoles in 150 mL of de-chlorinated water using an ultrasonic homogenizer (to ensure specimens were rapidly killed) (Hagman and Shine 2008). This solution was subsequently filtered using a coarse porosity (25 $\mu$ m particle retention)

crepe fluted filter paper to remove all tadpole tissues, thereby minimizing any potential nutritional enhancement from the addition of the conspecific cue solution to the tadpoles' diet. Control solutions consisted of 150 mL of de-chlorinated water that had similarly been homogenized and filtered for consistency. The entire 150 mL of each solution was added to the separate diet preparations intended for each of the treatment groups. The conspecific cue solution was provided to each tadpole with their food to ensure a strong association between the food and the chemical cues. Tadpoles were fed to satiation throughout the study and dietary rations for individual tadpoles were increased uniformly across treatments through experimental progression in response to increased tadpole feeding.

According to Relyea (2004) the magnitude of tadpole growth response should depend on food availability. Consequently, here we used a high quality diet and the effects of competition were negated to promote growth and development. Such conditions were used to identify whether the inclusion of the chemical cues would deter tadpoles from foraging on a profitable diet.

Tadpoles were removed from the study when they achieved Gosner (1960) stage 41 of development (initiation of metamorphosis) and were subsequently euthanized using an overdose of a buffered TMS (tricaine methanesulfonate) solution. Final measurements of mass, body length (BL), body width (BW), maximum tail fin depth (TFD), maximum tail muscle width (TMW), and time to metamorphosis (TTM) were recorded. Measurements of each trait were produced as mean values of 3 replicate measurements per tadpole. Morphometrics were measured using digital calipers, and mass was measured using a Sartorius digital balance, having observed measurement errors of  $\pm 0.02$  mm and  $\pm 0.002$  g, respectively.

### ***3.3.3 Stable Isotope Analyses***

All tadpole specimens were eviscerated, frozen, and subsequently freeze dried in a Labconco Corp. Freezone® freeze drier for approximately 24 hr in the Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada. Freeze dried whole body tadpoles were pulverized to a fine powder, weighed and packaged at National Hydrology Research Center of Environment (NHRC), Saskatoon, SK, Canada. Homogenized whole body samples were used to compensate for differences in fractionation between animal tissues (Biasatti, 2004). Dry powder samples were weighed to approximately 0.100 mg, and packaged in Elemental Microanalysis Ltd. 5 x 3.5 mm tin capsules. Samples were subsequently submitted for C and N stable isotope mass spectrometry analysis to the Stable Isotope Hydrology and Ecology Research Laboratory at NHRC, and the Stable Isotope Laboratory of the Department of Soil Science, U of S. Previous lipid proportions of wild wood frog tadpoles were consistently below 5%. Therefore, no lipid processing of samples submitted for isotope analysis was warranted (Post et al., 2007). Values of stable isotopes ( $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ ) were found as the deviance ( $\delta$ : delta) of the ratio of heavy to light isotopes (i.e.,  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ) within a sample, to that of an international standard (VPDB, AIR), and expressed in parts per thousand (‰) (Biasatti, 2004). Stable isotope values were quantified and reported as described in Jardine et al. (2003). Measurement error based on replicate measurements of in-house organic standards (egg albumen, whale baleen) was estimated to be  $\pm 0.1$  and  $\pm 0.2\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , measurements, respectively.

### ***3.2.4 Statistical Analyses***

A multivariate analysis of variance (MANOVA) was used to compare time to metamorphosis, mass, and morphometric values of tadpoles between treatments.



Subsequent *post-hoc* analyses were conducted to explore potential causes of results from initial analysis. A conservative testing procedure was adopted by adjusting significance levels using the Bonferroni method ( $\alpha = 0.01$ ) to reduce the risk of committing a type I error (Sokal and Rohlf 1995). An analysis of covariance (ANCOVA) was used to compare tadpole body width adjusted for differences in snout-vent length. Additionally, patterns in body growth of tadpoles was tested by comparing the slopes of regression lines against the expected slope for isometric growth (slope of 1) using one-sample *t*-tests of regression lines for body width on body length.

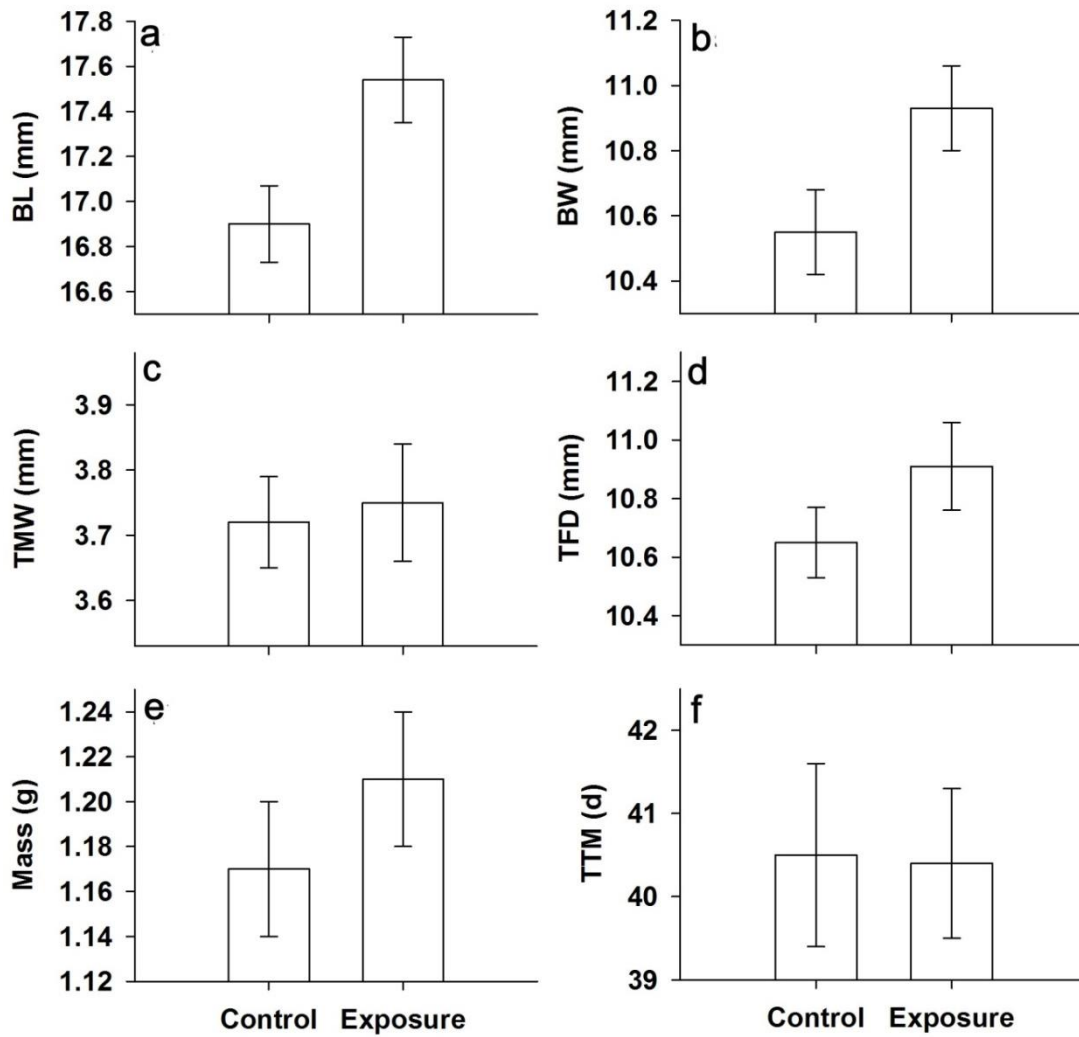
To test for differences in foraging, a total of 23 tadpoles (12 control and 11 exposed) were randomly selected and analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Stable isotopic ratios of tadpoles were compared between treatments, using a MANOVA. Observed values were subsequently compared to the expected  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values based on the ratio of diet provided (approximately  $\delta^{13}\text{C} = -25.6\text{‰}$  and  $\delta^{15}\text{N} = 5.9\text{‰}$ , based on a 4:1 ratio of brine shrimp to rodent pellet). Proportions of assimilated diets were subsequently assessed using Stable Isotope Sourcing using Sampling (SISUS) implemented in the SISUS package (Erhardt, 2007) for R, version 2.13.2 (R Development Core Team, 2010).

Mortalities were excluded from all analyses. Linearity was assessed among morphometric traits, data was log-transformed where it violated parametric assumptions, and statistical outliers were removed. All analyses, other than SISUS were performed in Systat (Wilkinson, 1998).

### **3.3 Results**

A total of 26 mortalities occurred throughout the experiment; 12 occurred in the control group and 14 in the exposure group. No aversion to foraging was observed among tadpoles at any point in the study.

Tadpoles exhibited significant difference in response to experimental treatments (Hotelling-Lawley Trace:  $F_{[6,56]} = 3.09$ ;  $P = 0.01$ ). Univariate  $F$ -tests indicate tadpoles exposed to injured conspecifics cues were significantly larger in body length ( $F_{[1, 61]} = 5.96$ ;  $P = 0.01$ ) with a mean ( $\pm$  SE) of 17.54 ( $\pm$  0.19) mm vs. 16.90 ( $\pm$  0.17) mm of control tadpoles. Exposed tadpoles also had significantly larger body width ( $F_{[1,61]} = 4.03$ ;  $P = 0.04$ ) having a mean ( $\pm$  SE) value of 10.93 ( $\pm$  0.13) mm relative to 10.55 ( $\pm$  0.13) mm of control tadpoles (Fig. 3.1).



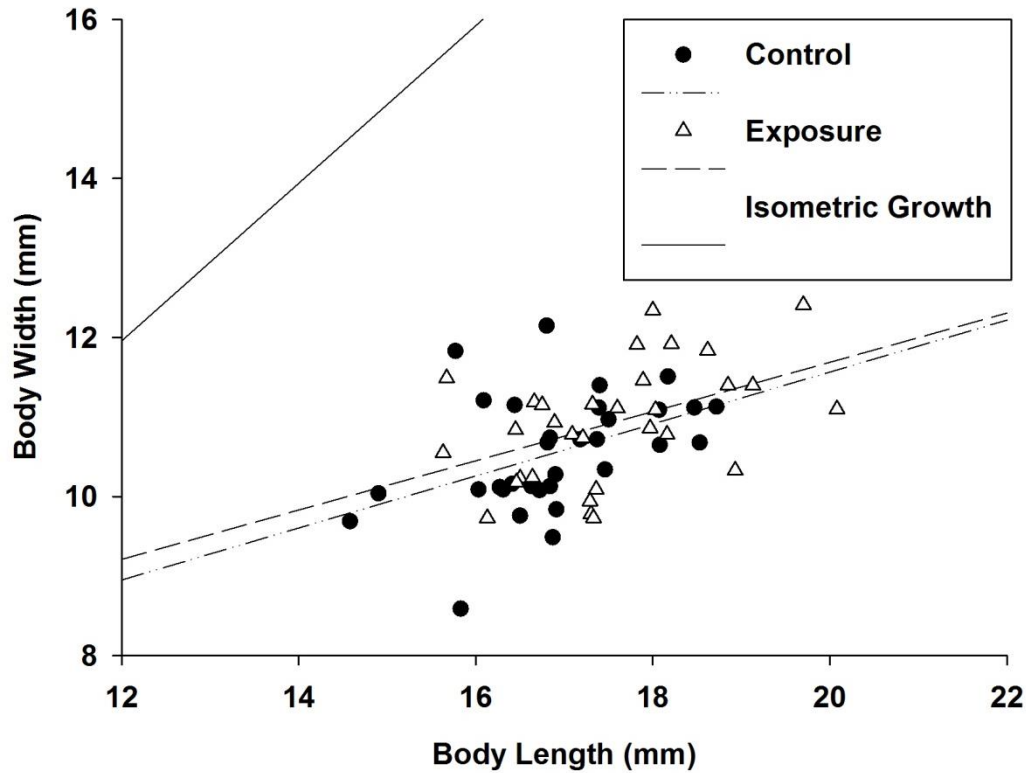
**Figure 3.1:** Tadpole morphometrics and time to metamorphosis between treatments. Comparison of mean ( $\pm 1SE$ ) tadpole (a) body length (BL), (b) body width (BW), (c) tail muscle width (TMW), (d) tail fin depth (TFD), (e) mass, and (f) time to metamorphosis (TTM) between control and exposure to injured conspecific cues treatments.

However, no significant differences were observed between experimental treatments in mass (mean  $\pm$  SE;  $1.21 \pm 0.03$  g vs.  $1.17 \pm 0.03$  g) or tail morphology (mean  $\pm$  SE; TFD:  $10.91 \pm 0.15$  mm vs.  $10.65 \pm 0.12$  mm; TMW:  $3.75 \pm 0.09$  mm vs.  $3.72 \pm 0.07$  mm) (all  $P > 0.05$ ; Fig. 3.1). Additionally, time to metamorphosis was not significantly different for tadpoles between treatments (mean  $\pm$  SE;  $40.5 \pm 1.1$  d vs.  $40.4 \pm 0.9$  d;  $P > 0.05$ ; Fig. 3.1) (Table 3.1).

**Table 3.1:** Univariate *F*-test. Comparisons between control and exposure treatment groups of time to metamorphosis (TTM), mass, and morphometrics: body length (BL), body width (BW), tail fin depth (TFD), and tail muscle width (TMW).

Source	SS	df	MS	<i>F</i>	<i>P</i>
TTM	0.00	1	0.00	0.00	0.99
Error	1.20	61	0.02		
Mass	0.01	1	0.01	0.67	0.42
Error	1.21	61	0.02		
BL	0.02	1	0.02	5.96	0.01
Error	0.22	61	0.00		
BW	0.02	1	0.02	4.03	0.04
Error	0.29	61	0.00		
TFD	0.01	1	0.01	1.79	0.19
Error	0.30	61	0.00		
TMW	0.00	1	0.00	0.04	0.85
Error	0.89	61	0.02		

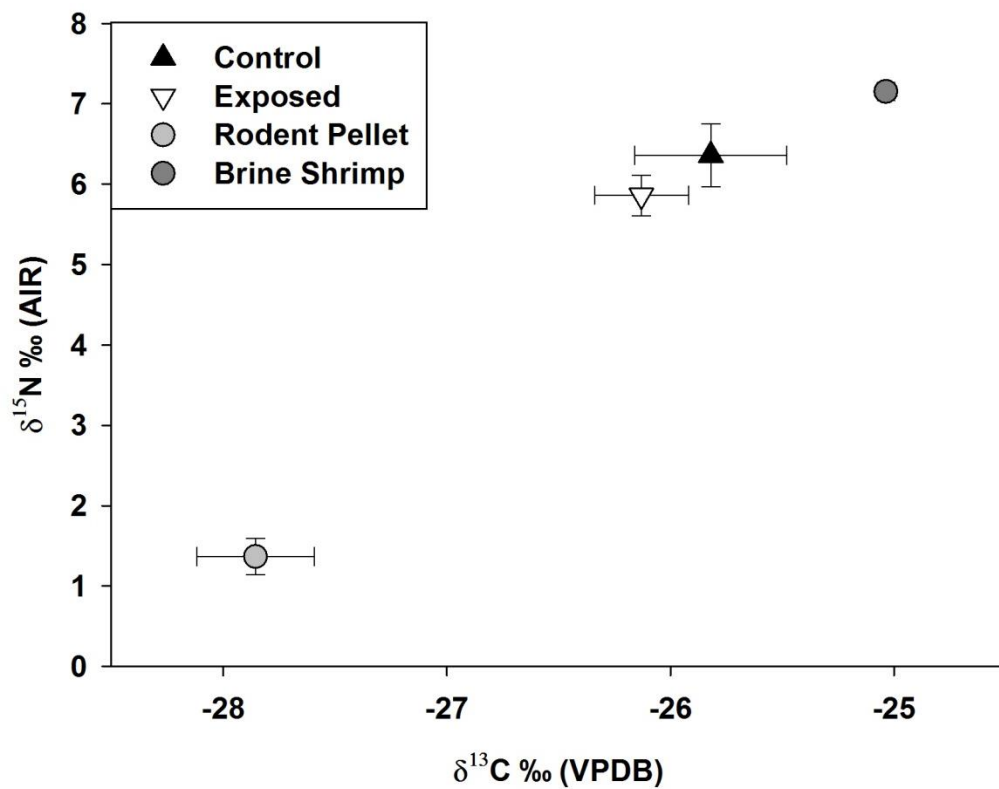
Further comparison of tadpoles between treatments identified no difference in the ratio of body width to body length between groups (equivalent slopes) and no difference in tadpole body width, when adjusted for differences in body length (ANCOVA:  $F_{[1,60]} = 0.95$ ;  $P = 0.33$ ; Fig. 3.2). Regression lines of body width against body length exhibited slopes significantly less than 1 for both treatment groups (control:  $t_{[31]} = -89.8$ ; exposed:  $t_{[30]} = -91.8$ ; all  $P < 0.001$ ), describing a negative allometric growth pattern. This suggests differences between treatments are due to greater overall body size in exposed tadpoles, and growth of body width was less than that of body length at this point in development.



**Figure 3.2:** Body growth of tadpoles. Comparison of negative allometric growth of body width (BW) and body length (BL) observed in tadpoles of the exposed (open triangles), and control (solid circles) treatments, relative to the regression line of typical isometric growth. Equations of regression lines of BL (X) adjusted BW (Y) of exposed tadpoles:  $Y = 5.49 + 0.31X$ ,  $r^2 = 0.21$ ; control tadpoles:  $Y = 5.03 + 0.33X$ ,  $r^2 = 0.18$ ; and of the isometric growth line  $Y = 0 + 1X$ ,  $r^2 = 1$ .

Analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values indicate that tadpoles in the two treatments did not differ in foraging or assimilation of dietary resources (Hotelling-Lawley Trace:  $F_{[1,19]} = 1.14$ ;  $P = 0.34$ ). Control and exposed tadpoles exhibited a mean ( $\pm$  SE)  $\delta^{13}\text{C}$  values of  $-25.8 (\pm 0.3)\text{‰}$  and  $-26.1 (\pm 0.2)\text{‰}$ , respectively, and mean ( $\pm$  SE)  $\delta^{15}\text{N}$  values of  $6.4 (\pm 0.4)\text{‰}$  and  $5.9 (\pm 0.2)\text{‰}$ , respectively. The majority dietary component, frozen brine shrimp ( $n = 6$ ) had mean ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $-25.0 (\pm 0.0)\text{‰}$  and  $7.2 (\pm 0.0)\text{‰}$ . The generic rodent pellet food ( $n = 6$ ) exhibited mean ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $-27.8 (\pm 0.3)\text{‰}$  and  $1.4 (\pm 0.2)\text{‰}$ . Assimilation of the mixed dietary resources was not significantly different from the

provided ratio, based on stable isotopic values, after Bonferroni correction (Control  $\delta^{13}\text{C}$ :  $t_{[10]} = -0.30$ ,  $P = 0.77$ ;  $\delta^{15}\text{N}$ :  $t_{[10]} = 1.53$ ,  $P = 0.16$ ; Exposed  $\delta^{13}\text{C}$ :  $t_{[10]} = -2.54$ ,  $P = 0.03$ ;  $\delta^{15}\text{N}$ :  $t_{[10]} = -0.50$ ,  $P = 0.03$ ). SISUS (Erhardt, 2007) models identified a range of ratios (4.3:1 to 4.7:1) that were similar to the 4:1 ratio of dietary resources provided. These results indicate that tadpoles foraged indiscriminately upon the provided diet, and did not preferentially consume or assimilate resources (Fig. 3.3).



**Figure 3.3:** Mean ( $\pm 1$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of tadpoles in control (no conspecific cues added) and exposed (conspecific cues added) treatments, and the two components of their mixed diet; brine shrimp and generic rodent pellet.

### 3.5. Discussion

For a variety of prey animals chemical cues of injured conspecifics elicit avoidance behaviour and are associated with reduced activity and foraging (Petranka, 1989; Schoeppner

and Relyea, 2005; Ferrari and Chivers, 2008; Fraker et al., 2009; Schoeppner and Relyea, 2009; Ferrari et al., 2010). Such a response improves survivorship by reducing exposure to potential threats and facilitating improved escape (Van Buskirk and McCullum, 2000; Relyea, 2004). In many tadpoles, alarm cues alone are known to induce dramatic behavioural responses, but long-term exposure may not induce morphological defences (Relyea, 2004; Hagman and Shine, 2008). However, alarm cues are known to induce adaptive changes in morphology in other taxa (Stabell and Lwin, 1997; Chivers et al., 2008), and the ability of prey to induce such changes is dependent on the frequency and intensity of risk. Hagman et al. (2009) observed that exposing cane toad tadpoles to alarm cues of crushed conspecifics reduced the size of metamorphs, and increased the size of the bufotoxin containing paratoid glands and the amount of the toxin bufalin produced by these toads. Conversely, the results of this study indicate exposed tadpoles exhibited an apparent increase in overall body size with no difference in time to metamorphosis, nor observed tail morphology relative to the control treatment.

My results are not consistent with the expected results from a predation risk perspective (e.g. Relyea, 2004). Tadpoles exposed to conspecific cues in this study also do not conform to the induced “bulgy” morphology, which describes tadpoles with relatively widened and deepened bodies relative to body length (Kishida and Nishimura, 2004). Tadpoles generally exhibit isometric growth (Strauss and Altig, 1992), and positive allometry is expected in induced morphological responses, such as “bulgy” tadpoles described by Kishida and Nishimura (2004). The negative allometric growth observed in tadpoles of both groups may reflect decreasing body width of tadpoles as they initiated dramatic metamorphic alterations occurring in tadpoles at this stage of development (Schiesari et al., 2009). Stable isotopic ratios indicate that exposed tadpoles did not exhibit the preferential foraging of dietary resources observed by Richter-Boix et al. (2007). Differences in body size may,

therefore have resulted from stimulation of increased foraging or improved nutrient assimilation of exposed tadpoles (Relyea, 2004; Relyea and Auld, 2004). A previous study by Ferrari et al. (2008) identified rapid degradation (within 2h) of larval wood frog alarm cues under natural conditions. Tadpoles would therefore have experienced repeated acute exposure through the duration of the study. Neuroendocrine responses to stress, leading to avoidance behaviour, may have been initially invoked (Crespi and Denver, 2004; Schoeppner and Relyea, 2005; Fraker et al., 2009; Schoeppner and Relyea, 2009), however, the exposure period may have been insufficient to sustain suppressed foraging. Conversely, this neuroendocrine response often stimulates appetite subsequent to expression of suppressed feeding and avoidance behaviour (Crespi and Denver, 2004). This response acts to increase foraging after a stress or risk has been removed to replenish energy stores (Crespi and Denver, 2004). This may have compensated for any initial suppression of feeding imposed upon tadpoles by acute exposure to these chemical cues. However, this does not account for the observed increased body size, or the absence of a reduction in developmental rate.

The discrepancy between the observed and expected results from a risk perspective may be explained by the interpretation of the cues tadpoles were exposed to. Relyea (2004) observed that tadpoles specifically adjust their behavioral and phenotypic responses to efficiently adapt to overall ecological risks (i.e. predation, and competition) and available resources (i.e. quality and quantity of food) to improve their survival. The specificity and/or duration of the tadpoles' response may also relate to the degree of the perceived risk, whereby animals may exhibit a much greater or prolonged response in situations of high-risk, and a lesser and/or shorter response under low risk situations (Lima and Bednekoff, 1999; Mirza et al., 2006; Ferrari et al., 2009). The observed morphological response of tadpoles does not correspond with typical responses of exposure to alarm cues, however, it does resemble observations from studies of increased intraspecific competition (Relyea, 2002;



Michel, 2012). Multiple studies have observed increased body size in wood frog tadpoles in response to high competition (Relyea, 2000; Relyea, 2002; Relyea, 2004; Relyea and Auld, 2004; Michel, 2012). Relyea (2002) observed that tadpoles exhibit similar responses regardless of whether tadpole density or food density was manipulated and therefore asserts that tadpoles assess levels of competition based on chemical cues. Tadpoles may identify increasing concentrations of innate chemicals (e.g. urine or feces) within the habitat as a sign of increased competition (Relyea, 2002). Relyea and Auld (2004) identified increased intestinal length associated with increased competition. The authors further suggest that this increased intestinal length may account for the larger body sizes of tadpoles under increased competition (e.g. Relyea, 2002; Relyea and Auld, 2004). It is possible that in my current study these chemical cues were present in the solution provided to tadpoles, and may have induced the observed responses.

However, results are not consistent among all studies of wood frog tadpoles. For example, results of this study and those of Relyea (2002) identified increased tadpole body width in response to high competition, while Michel (2012) observed no such increase in body width in the same species. Similarly, Relyea and Auld (2004) observed increased gut length in response to increased competition, and Michel (2012) observed no such difference. Relyea (2002) and Michel (2012) both observed decreases in tail morphometrics, which were not observed in this study. Relyea (2002) also observed reduced growth rate (g/d) as competition increased, while the results of this study suggest no such difference between exposed and control treatments. These differences may simply reflect the plasticity of wood frog response observed by Relyea (2004). He suggests that tadpoles respond in accordance to ecological context (Relyea, 2004). In this study it is possible that tadpoles identified a relatively low-risk situation, and focused on a perceived risk of intraspecific competition. The observed differences in body size between the exposed and control groups may have been the

result of increased intestinal length in exposed tadpoles as suggested by Relyea and Auld (2004). In the absence of competition or any difference in diet, the cost of the increased intestinal length may have off-set any benefit this adaptation provided to the exposed tadpoles, therefore negating any expected increase in mass.

My results suggest tadpoles may shift their perception of, or focal response to, specific risks. I suggest that early in the experiment tadpoles may have responded to chemical cues as an indication of risk until these cues degraded (i.e. Ferrari et al., 2008). The remaining chemical cues, combined with metabolic waste released from experimental tadpoles, would then persist within the experimental container and possibly be interpreted as cues of increasing competition (i.e. Relyea, 2002). After prolonged acute exposures to these chemical cues in the absence of a threat, tadpoles ceased responding to the risk cues and began to respond to the putative cues of increasing competition. The ability of tadpoles to correctly assume risk level is paramount to their immediate survival; to shift focus from risk of predation and towards increasing competition suggests that tadpoles identify this as a looming threat to survival or fitness. The results of this study indicate that tadpoles are capable of assessing multiple, possibly conflicting ecological conditions and subsequently respond accordingly to efficiently mitigate risks. Although further investigation is necessary, these observations provide further insight into the risk assessment of tadpoles and may highlight the evolutionary pressures that act towards development of response plasticity.

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## **Chapter 4: Time to feed: how diet, competition, and experience may influence feeding behaviour and cannibalism in wood frog tadpoles, (*Lithobates sylvaticus*).**

The contents of Chapter 4 have been published manuscript in Current Zoology (2014) **60**: 571-580 under joint authorship of Keith A. Hobson and Douglas P. Chivers. The contents have been modified from their originally published version to reflect comments provided by the examining committee, to reflect the format of the overall thesis, and Figure 4.1 has been modified from its original version into a series of bar graphs.

### **4.1 Abstract**

Wood frog (*Lithobates sylvaticus*) tadpoles develop in temporary wetlands where high population densities can force tadpoles into aggregations that intensify competition and can lead to cannibalism. However, chemical alarm cues released from injured conspecifics could also dissuade cannibalism. The purpose of this study was to test mechanisms that may influence cannibalistic behaviour. I tested whether the tendency of tadpoles to consume conspecifics would increase with the presence of competition and/or cues of profitable diets. Tadpoles placed in 1L experimental containers were tested for feeding initiation times of multiple diets, including conspecific tissues and conspecific tissues combined with chemical cues from alternative diets (brine shrimp and cornmeal). Tadpoles were tested in the presence and absence of a competitor, and at multiple times, over the course of the study. Tadpoles exhibited an altered response to diets over time; however, the presence of a competitor reduced response times to all diets, including conspecific tissues. Similarly, the presence of specific diets also reduced the response time of tadpoles to conspecific tissues. These results

suggest competition among feeding tadpoles could result in aggressive behaviour, leading to indiscriminate predation and cannibalism.

## **4.2 Introduction**

Tadpoles are non-reproductive, continuously feeding stages of anurans (Audo et al. 1995) that have been described as being “essentially eating machines” Wells (2007). Tadpoles often develop in wetlands of unknown duration and must be capable of rapidly adjusting to fluctuating conditions (Audo et al., 1995; Bleakney, 1958; Michel, 2012; Relyea, 2002; Relyea, 2004; Wellborn et al., 1996). Such ephemeral habitats are inherently unpredictable in terms of food quality and availability, population density, environmental conditions, and community composition (Babbitt and Meshaka, 2000; Bleakney, 1958; Newman, 1987; Wellborn et al., 1996; Wilbur, 1980). Simply put, the potential combinations of hazards presented to tadpole survival in such habitats are numerous. Therefore, profitable resources should be exploited as rapidly as possible. However, tadpoles must also be discerning consumers, balancing the potential benefits of resource utilization with lurking risks (e.g. predation or injury, competition, disease, etc.) (Relyea, 2002; Relyea, 2004; Richter-Boix et al., 2007).

Cannibalism is a relatively common and theoretically ideal diet by composition, which can potentially reduce competition. Cannibalism represents a highly profitable foraging strategy because conspecific tissues contain all essential nutrients, presumably in the appropriate proportions for growth and development (Greenstone, 1979; Meffe and Crump, 1987; Wildy et al., 1998). Such a diet can improve growth, development, and/or survivorship of individuals experiencing dietary stress resulting from reductions in quality and/or quantity of dietary resources (Fox, 1975; Meffe and Crump, 1987; Babbitt and Meshaka, 2000). Conversely, cannibals put themselves at risk of retaliation from their putative victims; they

may increase their risk of contracting disease, and can reduce their fitness by killing their kin (Bolker et al., 2008; Fox, 1975; Parris et al., 2005; Wildy et al., 1998; Wildy et al., 2001; Williams and Hernández, 2006). Additionally, chemical alarm cues released from injured conspecifics should trigger an innate anti-predator behaviour in proximal tadpoles, which could deter potential cannibals from feeding upon these tissues (Chivers and Smith, 1998; Ferrari et al., 2008; Moir and Weissburg, 2009).

Wood frog tadpoles (*Lithobates sylvaticus*) are highly efficient predators upon many species of amphibians, including conspecifics, during their vulnerable larval stages (Petranka et al., 1994; Petranka et al., 1998; Petranka and Thomas, 1995). However, wood frog tadpoles lack the specialized structures typically associated with highly carnivorous tadpole species (i.e. enlarged jaws, modified beaks and teeth, and shortened digestive tracts) and vulnerable conspecifics are increasingly capable of evading cannibalism over time (Crump, 1983; Petranka and Thomas, 1995; Wassersug, 1980; Wassersug et al., 1981).

Petranka and Thomas (1995) suggest that the evolution of explosive concurrent breeding among wood frogs may have been positively influenced by the efficiency of wood frog tadpoles to cannibalize vulnerable conspecifics. Indeed, synchronized explosive breeding can reduce the risk of cannibalism by limiting differences in size and development between individuals (Crossland et al., 2011; Petranka and Thomas, 1995). Additionally, food availability and conspecific density are influential factors to the expression of intraspecific aggression and cannibalistic behaviour (e.g. Collins and Cheek, 1983; Walls, 1998; Wildy et al., 2001). Babbitt and Meshaka (2000) suggest that cannibalism among free-feeding tadpoles is an adaptation to low food quality and/or food abundance rather than one of pond drying, due to the observation of prolonged larval development among tadpoles fed a high-quality diet and conspecifics tissues. Individuals engaged in cannibalism are often older tadpoles that

attack eggs and newly hatched larvae (Petranka and Thomas, 1995). It is unclear why cannibalism is not more common and pervasive throughout tadpole populations.

The goal of my study was to provide insights into the proximate causes of cannibalistic behaviour in larval amphibians. In this experiment I set up feeding trials whereby the risks and energy expenditures of feeding are minimized and nutritional benefits of diets are variable. I tested the relative feeding response of tadpoles to conspecific tissues and the proximate mechanisms that may encourage tadpoles to consume them. Specifically, I tested the hypothesis that tadpole response to feeding upon conspecific tissues could be encouraged by the presence of specific dietary cues and competitors, and may change through ontogeny. Typically, exposure to injured conspecific tissues should elicit predator avoidance behaviour in tadpoles causing them to avoid feeding on these tissues. However, I predicted that the presence of competitors and/or chemical cues from profitable diets may alter the response of tadpoles to conspecific tissues, potentially encouraging them to feed more rapidly upon them. Additionally, tadpoles may become less fastidious over time and thus initiate feeding more rapidly as a result of an ontogenetic change in feeding preference or acquired experience.

## **4.3 Materials and Methods**

### **4.3.1 Field Collections**

Wood frog eggs were collected from multiple disjunct ephemeral wetlands near Dalmany SK, Canada. Egg masses were transported to the University of Saskatchewan, and maintained in four 38L glass aquaria filled with room temperature de-chlorinated water. Tadpoles in all stock aquaria were maintained at approximately the same densities, and all were within the range of natural population densities observed by Biesterfeld et al. (1993).

While density was equally reduced in all aquaria throughout the experiment, the densities of tadpoles in each tank were consistently greater than 5/L.

Tadpoles maintained in stock aquaria received water changes seven days a week and were fed immediately after all water changes were completed. Water quality (i.e. pH, NH<sub>3</sub>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, Cl) of experimental containers was assessed a minimum of once a week within 24 hours of a feeding using commercially available aquarium test strips. Experiments were performed under a controlled 14:10 h (light:dark) photoperiod. Mean ( $\pm$  SE) water temperatures for experimental containers were 17.4° C ( $\pm$  0.2) through the course of the experiment. Prior to testing, tadpoles were fed a 1:1 mixed diet of frozen brine shrimp and cornmeal by mass. Each diet was added individually with a 5 min lag period between the addition of each diet, and the order in which each diet was delivered was rotated for each feeding to allow tadpoles to identify individual dietary cues and associate them with each diet.

#### **4.3.2 Experimental Procedures**

Tadpoles in stock aquaria were maintained exclusively on a 1:1 mixture of brine shrimp and cornmeal by mass. However, tadpoles were observed to cannibalize conspecifics throughout the study in these stock aquaria. Tadpoles were fed for 3 d prior to initial testing to provide equal exposure to experimental diets and ensure all tadpoles had initiated free-feeding. Approximately 24h prior to testing, tadpoles were haphazardly selected from among the four stock aquaria and transferred into a fifth, clean 38L glass aquarium for 24h prior to experimental testing. Putting tadpoles into a clean container without food was done to increase the motivation to feed in the subsequent experiment. Though tadpoles were not provided food during this period their lower digestive tracts appeared relatively full and

therefore they were not starved. Wood frog tadpoles are corprophagous and hence may have been feeding upon deposited fecal material.

Five experimental diets were utilized to test the time it took tadpoles to initiate feeding: 1) a high protein diet ( $54.9 \pm 1.6\%$  dry protein) of frozen brine shrimp (BS); 2) a low protein diet ( $6.6 \pm 0.2\%$  dry protein) of cornmeal (CM); 3) a proxy cannibal diet of macerated conspecific tissues (CAN), which has similar protein content as BS ( $53.6 \pm 1.6\%$  dry protein); 4) macerated conspecific tissues combined with the chemical cues from frozen brine shrimp (CAN + BS cue); and 5) macerated conspecific tissues mixed with chemical cues from cornmeal (CAN + CM cue). Granules of CM were ground using a small electric food grinder producing variety in CM granule size from the typical  $\leq 1\text{mm}$  granules to a fine powder. This was done to provide a viable food source that is easy to find, and that would also have particulates that could be suspended, at least temporarily, in water and produce cues that could be detected by tadpoles. All estimates of dry protein content of diets were calculated by multiplying the %N values of dietary samples from a previous study by 6.25 as described by Sterner and Elser (2002).

Conspecific tissues were produced by physically euthanizing tadpoles via pithing followed by rapid maceration of the tissues, which were subsequently sealed in food quality plastic containers and rapidly frozen and stored until required for testing purposes. Tadpoles used to produce these tissues were randomly selected from the general stocking tanks at various times throughout the experiment. This was done to ensure that conspecific tissues consisted of tadpoles at various stages of development over time, to ensure gut loaded tadpoles had consumed a similar diet as tested tadpoles, and to provide a constant and relatively fresh source of tissues.

Chemical cues from BS and CM were produced by initially soaking 20g of each diet in 250mL of de-chlorinated water for approximately 30min. This solution was subsequently

filtered using a coarse porosity (25µm particle retention) crepe fluted filter paper to remove all tissues, thereby minimizing any potential nutritional enhancement from the addition of the cue solution to these two experimental diets of conspecific tissues. BS and CM were used as novel control diets of high and low quality, respectively. Conspecific tissues were used to test cannibalistic feeding, and chemical cues from BS and CM individually combined with conspecific tissues were used to test whether the presence of either of these dietary cues could influence tadpoles to initiate feeding upon an otherwise cannibalistic diet.

For each dietary treatment 20g of the respective diet was soaked in 250mL of de-chlorinated water or a cue solution for a minimum of 10min prior to testing. Each dietary treatment was tested on 25 replicates of individual tadpoles, and 25 pairs of tadpoles to observe the effects of the presence or absence of a conspecific on feeding. Tadpoles were tested at three different time periods approximately 10d apart. The initial testing period (Test 1) took place 3d after the initiation of free-feeding in when tadpoles were at Gosner (1960) stage 25, the second testing period (Test 2) began when tadpoles were between stages 25-28, and the final testing period (Test 3) took place when tadpoles were between stages 30-32. This was done to determine if there was a temporal shift in feeding response as a result of an ontogenetic change in feeding or increased experience with the diet. Effects resulting from ontogenetic shifts should be minimized as all tadpoles were approximately within the same “pre-metamorphosis” phase of development (Etkin, 1968). The order of treatment testing was assigned randomly and tadpoles were haphazardly selected for testing. Tadpoles, either individually (competitor absent) or in pairs (competitor present) were acclimated in 1L containers for a minimum of 10min prior to testing.

Introduction into a foreign habitat would inevitably create initial hesitation in tadpoles to initiate feeding. However, since tadpoles are continuous feeders and were limited in their feeding capacity for a period of 24hr, feeding initiation times were used as a measure of



tadpole willingness to feed in any given treatment combination. Dedicated 3mL syringes were filled with a specific experimental diet and slowly injected into the tadpole's container over approximately 5s below the water's surface, allowing dispersal of food and cues while minimizing disturbance to the tadpole(s). Diets were agitated immediately prior to filling syringes to ensure any putative cues were available in solution. After an experimental diet was injected, a digital stopwatch was started and tadpoles were closely observed to identify feeding initiation time, with a maximum observation time set at 20min. Initiation of feeding was defined as the observation of tadpoles' active ingestion visibly identifiable portions of food. Once a tadpole began feeding the timer was stopped and the time to initiate feeding was recorded.

Where tadpoles were tested in pairs, the time it took for one of the two tadpoles to begin feeding was the recorded time. This testing procedure was developed in order to characterize the multiple factors that may influence the relative feeding disposition of tadpoles towards the experimental diets, while mitigating the probabilistic effects that necessarily accompany an increase in density. Tadpoles typically settled into resting positions near the surface of the water, or occasionally at the bottom of the containers, by the end of the 10min acclimation period. The experimental container was sufficiently large that tadpoles resting at the surface were separated from the injected food at the bottom of the container by a depth of approximately 82mm (up to 20 times tadpole body length, depending on tadpole age). This meant that in the majority of cases tadpoles had to actively move to the food source to initiate active feeding. The relatively low container volume was selected to limit the dilution effect upon dietary and conspecific chemical cues, and foraging area was sufficiently small as to limit tadpole search period. Trials where the initiation of active feeding was inconclusive were repeated with previously untested specimens. Tadpoles were never used in more than one trial, and each specimen was euthanized through submersion in a buffered

overdose solution of TMS (tricaine methanesulfonate) immediately after each trial was completed.

#### **4.3.3 Statistical Analysis**

A 2 x 3 x 5 factorial analysis of variance (ANOVA) was used to test for differences in feeding initiation times of tadpoles among the five experimental diets, for single and paired tadpoles, and among three testing periods. All pairwise comparisons were performed using *post hoc* Tukey HSD tests. Subsequently, a two-way multivariate analysis of variance (MANOVA) was performed to test the effects of density and testing period on the feeding initiation times of tadpoles fed each of the experimental diets. Finally, three separate one-way ANOVAs were used to test for differences among feeding responses of tadpoles to diets at each of the testing periods with post hoc Tukey HSD pairwise comparisons. A conservative testing procedure was adopted where the experiment-wise error was Bonferroni corrected to  $\alpha = 0.01$ , to reduce the risk of committing a type one error (Sokal and Rohlf, 1995). Statistical outliers were removed where identified by the statistical software. All analyses were performed in Systat (Wilkinson, 1998).

#### **4.4 Results**

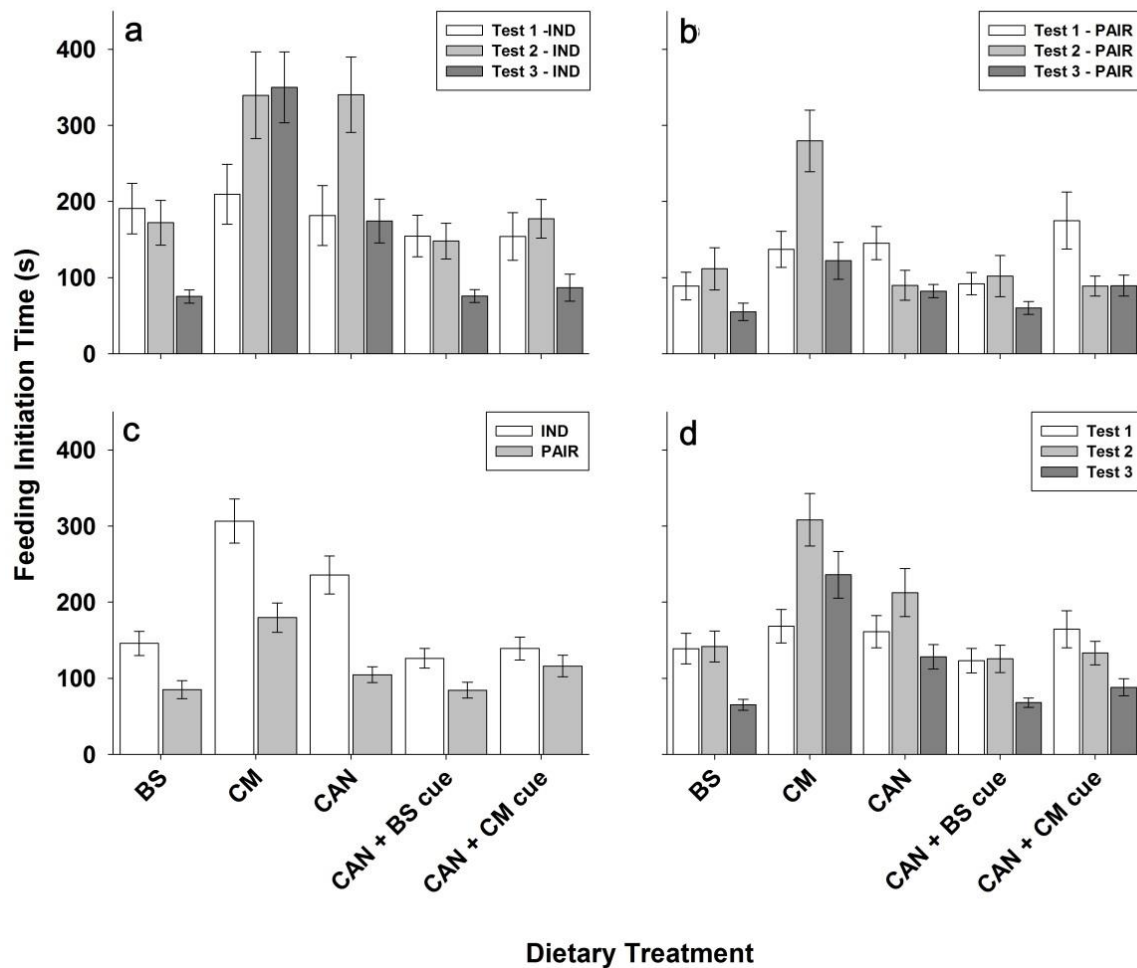
Tadpoles in stock aquaria were casually observed to form large feeding aggregations around patches of food resources, often within 30s of either diet being offered. Aggression was commonly observed among competing individuals, which was on multiple occasions observed to have led to cannibalistic behaviour. Cannibalistic behaviour was initially observed in stock aquaria within 8d of hatching, and within 2d of initiating free-feeding, at stage 25 of development. The initial observation of cannibalistic behaviour involved a relatively larger individual attacking a smaller individual from above and behind. The victim

was attacked, while feeding, from an aggressor who attacked from above using an attack similar to that described by Petranka and Thomas (1995). The attacker assumed a head-down position aimed at the victim and repeatedly bit near the dorsal posterior end of the victim's body propelling itself at the victim with thrusts from its tail (Petranka and Thomas, 1995). Similar observations were described by Caldwell and Araújo (1998) in interspecific predation between *Dendrobates auratus* (prey) and *Rana warschewitschii* (predator), and between *D. castaneoticus* (predator) and *Epipdeobates femoralis* (prey). Additional tadpoles rapidly began joining the original aggressor in frenzied feeding upon the dead or dying victim, similar to the situations described by Petranka and Thomas (1995) and Crump (1986). In the feeding melee up to five or six individuals were observed swarming the victim until the body was consumed. Similar observations were made additional times throughout the study.

Through experimental testing tadpoles exhibited significant difference in the time they required to begin feeding among the experimental diets ( $F_{[4,697]} = 22.4$ ;  $P < 0.001$ ). Overall, tadpoles exhibited significantly slower mean ( $\pm$  SE) feeding initiation times when fed CM ( $239.5 \pm 17.8$ s) than when provided any other diet (all  $P < 0.001$ ). There was no difference in the overall mean ( $\pm$  SE) feeding initiation times among tadpoles provided BS ( $115.6 \pm 10.2$ ), CAN + BS cue ( $105.5 \pm 8.5$ s), and CAN + CM cue ( $127.6 \pm 10.3$ s) (all  $P > 0.5$ ); nor was there any difference in overall times between tadpoles provided CAN ( $167.4 \pm 14.1$  s) or CAN + CM cues ( $P = 0.1$ ). However, tadpoles fed BS and those fed CAN + BS cues both began to feed more rapidly than those fed CAN alone (both  $P \leq 0.01$ ). Additionally, tadpoles typically initiated feeding more rapidly overall when a competitor was present than they did when tested individually ( $F_{[1,697]} = 51.8$ ;  $P < 0.001$ ; Fig 4.1a-c).

Tadpoles also exhibited a significant difference in overall feeding initiation times among the testing periods ( $F_{[2,697]} = 14.9$ ;  $P < 0.001$ ). Tadpoles exhibited significantly faster overall mean ( $\pm$  SE) feeding initiation in the final test period (Test 3:  $117.1 \pm 8.5$ s) than those

in both the initial testing period (Test 1:  $150.4 \pm 9.2$ s;  $P = 0.01$ ) and the second testing period (Test 2:  $183.4 \pm 11.9$ s;  $P < 0.001$ ). However, tadpoles tested at the second testing period required significantly longer to initiate feeding than tadpoles tested in the initial testing period ( $P = 0.03$ ; Fig. 4.1d).



**Figure 4.1:** Comparison of tadpole feeding initiation times to experimental diets. Differences in mean ( $\pm$ SE) feeding initiation times of tadpoles among individuals (a) and in pairs (b) at each test period; between pooled values of individuals and paired tadpoles across testing periods (c); and among testing periods using pooled values of individual and paired tadpoles (d).

Multivariate testing identified significant overall differences in feeding initiation times between individual tadpoles and those paired with a competitor (Hotelling-Lawley

Trace:  $F_{[5,140]} = 8.9$ ,  $P < 0.001$ ). Results of univariate  $F$ -tests indicate the effect of a present competitor (PC) was typically to significantly reduce response time of tadpoles relative to those fed in the absence of a competitor (AC). Significant reductions to overall mean ( $\pm$  SE) feeding initiation times were observed for tadpoles fed BS (PC:  $85.2 \pm 11.8$ s; AC:  $145.9 \pm 15.9$ s;  $F_{[1, 144]} = 10.2$ ;  $P < 0.005$ ), CM (PC:  $179.7 \pm 19.3$ s; AC:  $306.5 \pm 29.0$ s;  $F_{[1, 144]} = 20.8$ ;  $P < 0.001$ ), and CAN (PC:  $104.7 \pm 10.4$ s; AC:  $235.7 \pm 24.8$ s;  $F_{[1, 144]} = 10.2$ ;  $P < 0.005$ ). Tadpoles fed CAN + CM cue exhibited no significance difference between presence and absence of a competitor (PC:  $116.1 \pm 14.1$ s; AC:  $139.1 \pm 14.9$ s;  $F_{[1, 144]} = 0.9$ ;  $P = 0.4$ ), nor did tadpoles fed CAN + BS cue (PC:  $84.4 \pm 10.5$ s; AC:  $126.2 \pm 12.9$ s;  $F_{[1, 144]} = 3.2$ ;  $P = 0.07$ ).

Multivariate tests also identified significant overall differences in tadpole response times among testing periods (Hotelling-Lawley Trace:  $F_{[10, 278]} = 4.9$ ,  $P < 0.001$ ). Univariate  $F$ -tests identified significant differences in mean ( $\pm$  SE) feeding initiation times for tadpoles fed brine shrimp (Test 1:  $139.8 \pm 20.1$ s; Test 2:  $141.8 \pm 20.4$ s; Test 3:  $65.1 \pm 7.3$ s;  $F_{[2, 144]} = 7.0$ ;  $P = 0.001$ ), conspecific tissues (Test 1:  $161.2 \pm 21.1$ s; Test 2:  $212.6 \pm 31.6$ s; Test 3:  $128.2 \pm 16.2$ s;  $F_{[2, 144]} = 7.9$ ;  $P = 0.001$ ), and conspecific tissues combined with brine shrimp cues (Test 1:  $123.3 \pm 16.0$ s; Test 2:  $125.5 \pm 17.9$ s; Test 3:  $68.0 \pm 6.1$ s;  $F_{[2, 144]} = 5.4$ ;  $P = 0.005$ ), conspecific tissues combined with cornmeal cues (Test 1:  $164.5 \pm 24.2$ s; Test 2:  $133.2 \pm 15.5$ s; Test 3:  $88.1 \pm 11.1$ s;  $F_{[2, 144]} = 8.4$ ;  $P < 0.001$ ); no significant differences were observed among testing periods for tadpoles fed cornmeal (Test 1:  $168.4 \pm 22.1$ s; Test 2:  $308.3 \pm 34.4$ s; Test 3:  $236.0 \pm 0.7$ s;  $F_{[2, 144]} = 1.9$ ,  $P = 0.1$ ).

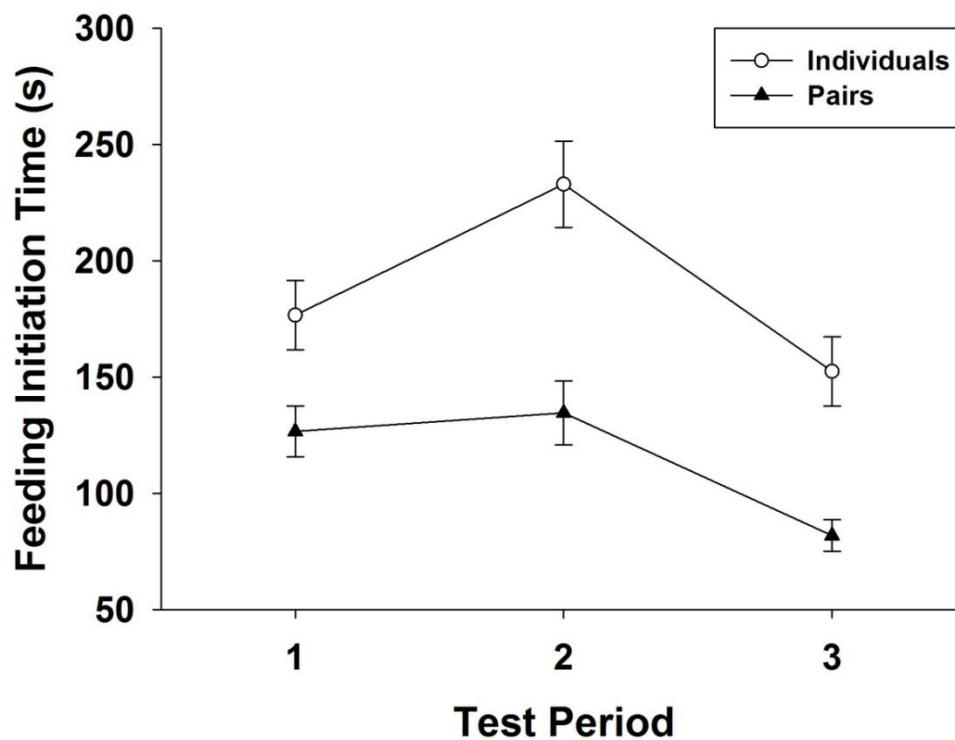
ANOVA testing indicate there was no significant differences in overall response times among tadpoles fed various diets at Test 1 ( $F_{[4,226]} = 0.9$ ;  $P = 0.5$ ). However, significant overall differences were observed among experimental diets in Test 2 ( $F_{[4,241]} = 9.5$ ;  $P < 0.001$ ) and Test 3 ( $F_{[4,236]} = 11.5$ ;  $P < 0.001$ ). Pairwise comparisons of tadpole response times

among diets in Test 2 indicate that tadpoles responded more rapidly to all diets relative to CM (all  $P < 0.001$ ) except for CAN ( $P = 0.05$ ). No other differences were observed in Test 2. In Test 3 tadpoles responded more rapidly to all diets relative to those fed CM (all  $P \leq 0.01$ ), and tadpoles fed BS responded more rapidly than those fed CAN ( $P = 0.04$ ). A complete summary of mean ( $\pm$  SE) values of feeding initiation times among all combinations of treatments are provided in Table 4.1.

**Table 4.1:** Summary of means and grand means ( $\pm$ SE) of tadpole feeding times for combination of diet, competition, and testing period.

Feeding Initiation Times (s)							
Test Period	Density	BS	CM	CAN	CAN + BS cue	CAN + CM cue	Total
1	1	190.7 $\pm$ 33.2	209.5 $\pm$ 39.4	181.5 $\pm$ 39.3	154.7 $\pm$ 27.4	154 $\pm$ 31.2	176.6 $\pm$ 14.9
	2	88.9 $\pm$ 18.2	137.2 $\pm$ 23.6	145.3 $\pm$ 21.8	91.9 $\pm$ 14.7	174.8 $\pm$ 37.5	126.6 $\pm$ 10.9
	Mean	139.8 $\pm$ 20.1	168.4 $\pm$ 22.1	161.2 $\pm$ 21.1	123.3 $\pm$ 16.0	164.5 $\pm$ 24.2	150.4 $\pm$ 9.2
2	1	172.1 $\pm$ 29.3	339.5 $\pm$ 56.9	340.4 $\pm$ 49.4	148.0 $\pm$ 23.5	177.5 $\pm$ 25.4	232.9 $\pm$ 18.6
	2	111.5 $\pm$ 27.7	279.6 $\pm$ 40.6	89.9 $\pm$ 19.6	101.9 $\pm$ 27.0	88.8 $\pm$ 13.0	134.6 $\pm$ 13.7
	Mean	141.8 $\pm$ 20.4	308.3 $\pm$ 34.4	212.6 $\pm$ 31.6	125.5 $\pm$ 17.9	133.2 $\pm$ 15.5	183.4 $\pm$ 11.9
3	1	75.1 $\pm$ 8.7	349.9 $\pm$ 46.5	174.3 $\pm$ 28.7	75.9 $\pm$ 8.5	86.9 $\pm$ 17.8	152.4 $\pm$ 14.9
	2	55.1 $\pm$ 11.6	122.2 $\pm$ 24.5	82.2 $\pm$ 8.7	60.1 $\pm$ 8.5	89.4 $\pm$ 13.7	81.8 $\pm$ 6.8
	Mean	65.1 $\pm$ 7.3	236.0 $\pm$ 30.7	128.2 $\pm$ 16.2	68.0 $\pm$ 6.1	88.1 $\pm$ 11.1	117.1 $\pm$ 8.5
Total	1	145.9 $\pm$ 15.9	306.5 $\pm$ 29.0	235.7 $\pm$ 24.8	126.2 $\pm$ 12.9	139.1 $\pm$ 14.9	187.3 $\pm$ 9.6
	2	85.2 $\pm$ 11.8	179.7 $\pm$ 19.3	104.7 $\pm$ 10.4	84.4 $\pm$ 10.5	116.1 $\pm$ 14.1	114.3 $\pm$ 6.4
	Mean	115.6 $\pm$ 10.2	239.5 $\pm$ 17.8	167.4 $\pm$ 14.1	105.5 $\pm$ 8.5	127.6 $\pm$ 10.3	150.1 $\pm$ 5.9

Significant interaction terms were observed among most of the tested independent variables. The most complex of which indicates that tadpole response to specific diets is dependent upon competitors and that this response differs depending upon the relative time period in which they are tested (diet x competitor x time period:  $F_{[8,697]} = 3.7$ ;  $P < 0.001$ ; Fig. 4.1a,b). Similarly, tadpole response to specific diets were not consistent through time (diet x time period:  $F_{[8,697]} = 3.2$ ;  $P < 0.005$ ; Fig. 4.1d), nor was the effect of competitor on tadpole response consistent among experimental diets (density x diet:  $F_{[4,697]} = 4.1$ ;  $P < 0.005$ ; Fig. 4.1c). However, overall tadpole response to competition was consistent among testing periods. The effects of density did not significantly change among testing periods (competitor x time period:  $F_{[2,697]} = 2.0$ ;  $P = 0.1$ ; Fig. 4.2), and this was shown again through multivariate testing (Hotelling-Lawley Trace:  $F_{[10,278]} = 1.4$ ,  $P = 0.2$ ).



**Figure 4.2:** Comparison of mean ( $\pm$ SE) tadpole feeding initiation times between pooled values of individuals and pairs of tadpoles across experimental diets.



## 4.5 Discussion

The results of this study demonstrate the plasticity inherent in the feeding responses of wood frog tadpoles. Such plasticity likely allows rapid adaptation to the varied ecological pressures of temporary wetland habitats (Faragher and Jaeger, 1998; Horat and Semlitsch, 1994; Relyea, 2004; Van Buskirk and Relyea, 2008). Tadpoles appear to efficiently adjust their feeding response to specific conditions involving dietary quality, competition, and potentially conflicting information from chemical cues. Additionally, tadpoles appear to change their responses to diet cues over time, possibly as a result of experience. These results provide insights into potential mechanisms that could increase cannibalistic tendencies within tadpole populations.

Relative responses of tadpoles generally conformed to those I expected for each of the experimental diets. Overall, tadpoles responded most rapidly to BS, CAN + BS, and CAN + CM, then CAN, and finally most slowly to CM. With regards to BS and CM, the response of tadpoles to these foods may be, at least in part, due to the protein content of each diet.

McCallum and Trauth (2002) found that tadpole growth and development increased with increasing dietary protein. BS is much higher in protein than CM, and this may be detectable to tadpoles through chemical cues and/or may be more palatable to tadpoles. However, protein alone does not explain the relative response of tadpoles to the CAN diet. CAN has similar protein content as BS, and is overall a potentially ideal diet, therefore CAN should at worst illicit a similar response as BS. What I observed, however, was a response that was slower than tadpoles fed BS, but faster than those fed CM. This suggests that while the nutritional value of CAN yields some benefit that can be detected by tadpoles, it also yields some threat or indicator of threat (i.e. alarm cues) that is also perceived by tadpoles deterring them from responding as rapidly as they might otherwise. Tadpole response to these putative

threats appeared to be mitigated by the presence of a competitor and/or cues from either a high or low protein diet.

Interestingly, while tadpole response time to the CAN + CM cues diet did not differ from tadpoles fed CAN tissues, they were also no different from tadpoles fed BS or CAN + BS cues. Tadpoles responded the slowest to CM out of all the experimental diets; therefore, it is unusual that the addition of cues from this diet would stimulate more rapid response times. However, since tadpoles fed CM did not respond favourably to this diet it is possible that tadpoles may be responding, in part, to the informational cues released from the conspecific tissues themselves. If tadpoles interpret these cues as an indication of threat from predation, then overall tadpole activity should be reduced as a method of predator avoidance (Ferrari et al., 2008). However, Horat and Semlitsch (1994) found that risk of predation did not reduce feeding activity, and Bridges (2002) observed that while tadpoles reduce their overall activity in the presence of a predatory threat, they significantly increased their time spent feeding. Therefore, if such cues were interpreted as a threat, an optimal response of tadpoles would be to identify a food source and initiate feeding as rapidly as possible. Alternatively, tadpoles may interpret these cues as an indicator of increased competition; this should initiate a similar response to that of a present competitor [Jefferson et al., 2013 (Chapter 3)]. This is supported in that tadpoles fed these diets did not significantly reduce their response time between trials in which tadpoles were tested individually or with a competitor. Jefferson et al. (2013; Chapter 3) suggest tadpoles interpret repeated exposure to cues from injured conspecific tissues as an indicator of increased competition.

The reduction in feeding initiation times where a second tadpole is present can be interpreted in two ways: 1) tadpoles are responding to putative competition; or 2) tadpoles perceive an increase in safety from potential lurking predators from greater density. While this experiment was not designed explicitly to directly answer which condition tadpoles are

responding to, the results seem to suggest that tadpoles are responding to competition. If tadpoles were responding to an improved sense of safety with increased density I would expect that tadpole response times should improve in all instances when tested in pairs, however this does not occur when tadpoles were fed CAN + BS cue and CAN + CM cue. Additionally, wood frog tadpoles are not typically gregarious; Waldman (1985) indicated that wood frog tadpoles only form polarized schools where densities are high and they are forced to aggregate around dietary resources (Wells, 2007). This suggests tadpoles should be more sensitive to the putative threat of competition than any potential benefit they may gain from increased density.

Overall feeding initiation times increased from Test 1 to Test 2, and decreased relative to both of the previous tests in Test 3. Results in Test 1 appear to represent the relative naïveté in early tadpole feeding and subsequent tests show a refinement in response to feeding conditions. This is supported by the analysis of overall feeding response within each test period; in Test 1 there was no overall difference in feeding initiation times among the proffered diets. These responses likely developed through the experiment as a result of ontogenetic changes (e.g. production of and/or sensitivity to informative chemical cues, increase size and mobility, etc.) and learned recognition of tadpoles to the inherent value of each diet and the risk of competition and tailored their responses over time to increase efficiency to each experimental situation. These results are consistent with learned foraging adaptations suggested by Peacor and Pfister (2006).

Peacor and Pfister (2006) observed intra-population size variation of tadpoles raised at high population densities resulted from phenotypic adaptations ('size-independent' factors) causing differences in foraging efficiency among individuals (Fuiman and Cowan, 2003). The effects of these traits become increasingly pronounced over time, causing size disparity among individuals within the population (Lomnicki, 1988; Peacor and Pfister, 2006;

Uchmanski, 1985). Schriever and Williams (2013) identified ontogenic shifts in the trophic positioning and specialization of individual wood frog tadpoles through their larval development. The observed niche specialization may result from increasing differentiation in foraging success among individual tadpoles, which corresponds with what would be expected based on the results of Peacor and Pfister (2006). Such phenotypic adaptations could be genetic or learned (Peacor and Pfister, 2006). Boldness is a common risk-related personality trait with a genetic basis that can influence foraging behaviour and subsequently alter the growth of animals (Coleman and Wilson, 1997; Peacor and Pfister, 2006; Wolf et al., 2007). In some species, or populations of species, boldness in a risky situation (e.g. threat of predation) is positively correlated with aggression towards conspecifics, including the development of cannibalistic behaviour (Bell and Stamps, 2004; Bourne et al., 2008; Huntingford, 1976). Additionally, where such traits provide advantages to individual growth, resulting in size variation, the potential for cannibalism may increase (Caldwell and Araújo, 1998; Crump, 1986; Faragher and Jaeger, 1998; Petranka and Thomas, 1995).

Social behaviour may also influence the tendency of cannibalism among tadpoles. Black (1970) indicated that cannibalistic tadpoles of *Spea bombifrons* were only observed where tadpoles were feeding in large aggregations. The author concluded that schooling behaviour was a form of protection against cannibalism. Conversely, Pfennig (1992a,b) suggested schooling may have encouraged opportunistic cannibalism in response to the dense aggregation of prey (Wells, 2007). Wood frog tadpoles rarely aggregate, except where high population densities force them to congregate in large numbers around food sources (Waldman, 1985; Wells, 2007). Tadpoles often exhibit aggressive behaviour to secure advantage in interference competition; such behaviour could escalate to cannibalism where tadpoles are aggregated around food sources (Faragher and Jaeger, 1998; Gromko et al., 1973; Savage, 1952; Waldman, 1985; Wells, 2007; Wilbur 1977). The results of this study

are consistent with previous observations that cannibalism is influenced by high population density and highlight the plasticity of wood frog tadpoles in their feeding response (Fox, 1975; Polis, 1981; Polis and Myers, 1985). The presence of dietary cues and/or the presence of competition may encourage tadpoles to feed more rapidly and less discriminately. In the context of high population density such responses provide mechanisms that may account for increased aggression among conspecifics. Aggression can escalate to cannibalism where tadpoles are forced into large feeding aggregations and intense competition. Additionally, where such predation occurs it appears to be immediately followed by frenetic feeding by large numbers of conspecifics possibly in response to cues released from the injured tadpoles (Crump, 1986; Petranka and Thomas, 1995).

The results of this study support my hypothesis that tadpole response to feeding upon conspecific tissues can be encouraged by the presence of chemical cues of dietary resources and/or the presence of a competitor. Additionally, tadpoles exhibited changes in their responses to diets over time, suggesting either a learned response and/or an ontogenetic shift to individual diets. These results provide a putative mechanism for density-dependant cannibalism that is often observed among larval amphibians (e.g. Collins and Cheek, 1983; Walls, 1998; Wildy et al., 2001). Such an innate sensitivity to competition may lead tadpoles to aggressive encounters where intraspecific predation is a possible outcome. Ontogenetic shifts towards consuming conspecific tissues at later developmental stages may act to encourage the predation of larvae from late-breeding individuals by established larvae as observed by Petranka and Thomas (1995). Such behaviour would limit population density, provide a profitable diet to established larvae, and may largely remove the offspring of late breeders which would be at greater risk of desiccation in ephemeral wetlands.

## 4.6 References

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## **Chapter 5: Shifty salamanders: transient trophic polymorphism and cannibalism within natural populations of larval ambystomatid salamanders.**

The contents of Chapter 5 have been published in *Frontiers in Zoology* (2014) **11**:76, under joint authorship with Maud C.O. Ferrari, Alicia Mathis, Keith A. Hobson, Eric R. Britzke, Adam L. Crane, Andrew R. Blaustein, and Douglas P. Chivers. Alicia Mathis, Eric Britzke, Douglas Chivers, and Maud Ferrari conducted field collections. Maud Ferrari, Eric Britzke, and Adam Crane participated in data collection and laboratory preparations. Maud Ferrari and Adam Crane aided in the data analysis. The order and format of the contents of this manuscript have been modified to reflect comments from the examining committee and the overall formatting of the thesis.

### **5.1 Abstract**

Many species of ambystomatid salamanders are dependent upon highly variable temporary wetlands for larval development. High larval densities may prompt the expression of a distinct head morphology that may facilitate cannibalism. However, few studies have characterized structural cannibalism (cannibalism facilitated by the expression of trophic polymorphism) within natural populations of larval salamanders. In this study I used two species of larval salamanders, long-toed (*Ambystoma macrodactylum*) and ringed salamanders (*A. annulatum*). Head morphometrics and stable isotopic values of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were used to identify the presence or absence of structural cannibalism. Weather conditions were also analyzed as a potential factor associated with the expression of cannibalistic morphology. Populations of salamander larvae did not consistently exhibit cannibalistic morphologies throughout collection periods. Larval long-toed salamanders

exhibited trophic polymorphisms when relatively lower precipitation amounts were observed. Larval ringed salamanders were observed to be cannibalistic but did not exhibit polymorphisms in this study. Structural cannibalism may be transient in both species; however in long-toed salamanders this morphology is necessary for cannibalism. Ringed salamanders can be cannibalistic without corresponding morphological features, however, the cannibal morph may prolong the viable time period for cannibalism. Additionally, weather conditions may alter pond hydroperiod, subsequently influencing head morphology and cannibalism.

## **5.2 Introduction**

Temporary wetlands are important habitats to the larval development of many species of amphibians (Wellborn et al., 1996). However, such habitats inevitably undergo pond drying imposing temporal and/or spatial limitations upon developing larvae (Wellborn et al., 1996; Griffiths, 1997). Wetland drying increases the risk of desiccation while simultaneously increasing larval density and potentially limiting food resource availability (Griffiths, 1997). These restrictions can force larvae into feeding aggregations, increasing the degree of intraspecific competition, and leading to aggression and intraspecific predation [Griffiths, 1997; Jefferson et al., 2014a,b (Chapters 4 and 2); Perdersen, 1993; Wildy et al, 2001].

Larval amphibians exhibit sensitivity to intraspecific competition and may express specific morphological and/or behavioural adaptations leading to improved foraging success and increased rate of development, which allows for larvae to survive and escape inclement conditions [Jefferson et al., 2014a,b (Chapters 4 and 2); Jefferson et al., 2013 (Chapter 2); Peacor and Pfister, 2006; Relyea, 2004; Richter-Boix et al., 2007; Wildy et al., 1999]. Many species of larval amphibians may exhibit morphological adaptations that could improve foraging success or alter the prey they consume (e.g., Michimae and Wakahara, 2001;



Pfennig, 1992; Reilly et al., 1992; Relyea and Auld, 2004). In larval ambystomatid salamanders such adaptations include enlarged feeding structures (i.e. jaws and teeth), which increase the prey size that larvae are capable of consuming, and in turn, increases their potential trophic niche width (Chivers et al., 1997; Walls et al., 1993). These structural polymorphisms have also been associated with cannibalistic behaviour because they facilitate the consumption of similarly-sized conspecifics (Blaustein et al., 2001; Nyman et al., 1993).

Here, I used two model species of larval salamanders (*Ambystoma macrodactylum* and *A. annulatum*) from populations previously observed to exhibit the “cannibalistic morphology” (Blaustein et al., 2001; Nyman et al., 1993). Both species are explosive breeders as they both produce large numbers of eggs over a short period of time and both utilize temporary wetlands for breeding and larval development (Petranka, 1998). However, these species differ in key aspects of their breeding strategy. Long-toed salamanders tend to breed synchronously, while ringed salamanders often breed over a period of a month or more (Petranka, 1998). This difference results in an age and size hierarchy within larval populations of ringed salamanders while populations of larval long-toed salamanders tend to be of similar age and are at least initially of similar size (Petranka, 1998).

Studies documenting trophic polymorphism have primarily shown cannibalistic behaviour under laboratory conditions (Nyman et al., 1993). However, Nyman et al. (1993) characterized the difference in head morphology of larval ringed salamanders between cannibals and non-cannibals based on gut content analysis from individuals in a natural population. The purpose of my study was to further test the linkage between cannibalism and morphology in two species that produce larvae exhibiting the “cannibalistic morph”. Additionally, since neither larval densities nor pond conditions are static in natural populations, I expect that expression of polymorphisms and/or cannibalism may be transient within these populations among years (Blaustein et al., 2001). Larval long-toed salamanders

from Oregon were tested using morphological, and carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopic data to compare differences in morphology with differences in trophic niche occupation. Larval ringed salamanders from Missouri were differentiated into cannibals and non-cannibals based on gut-content analysis and compared for differences in head morphology and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Climate data for collection sites were also used to identify weather patterns that may have influenced pond condition and subsequently the ecology of salamander larvae.

## **5.3 Materials and Methods**

### **5.3.1 Field Collections**

#### *Long-toed Salamanders*

Long-toed salamander larvae (*A. macrodactylum*) were collected with net sweeps performed from along the pond shoreline at two sampling periods in June and August 2007, and in July and August 2008 from an ephemeral montane pond located at an altitude of 1951 m above sea level in the central Cascade Mountains, 24.2 km south of Sisters, Deschutes County, Oregon. All larvae were physically euthanized by pithing, measured for head and body morphometrics with vernier calipers (to 0.1 mm), and frozen. Salamander larvae were measured for head length (HL; tip of snout to attachment point of first pair of gills), maximum head-width (MHW; width across the head at its widest point), snout-vent length (SVL; length from tip of the snout to the anterior end of the vent), and pre-ocular head width (PREHW; width across the head through bisecting line through the external nares). Data for PREHW of salamanders collected in August 2007 was lost and was therefore estimated from regression analysis of these characteristics against SVL from all other long-toed salamanders used in this study. Gut content analysis of these specimens was not possible due to the

physical degradation that occurred from frozen storage. Specimens were delivered to the University of Saskatchewan in August, 2010.

Climate data of the collection area (near Sisters, Oregon) for 2006 to 2008 were obtained from the Oregon Climate Service, Oregon State University, Corvallis, Oregon, USA. Precipitation and temperature data from September prior to sampling periods to the end of each sampling period (i.e. September 2006 – August 2007 and September 2007 – August 2008) was selected to characterize differences in weather conditions between sampling years. Extended weather data were included due to the potential influence of fall and winter precipitation on the hydrologic condition of the pond.

### *Ringed Salamanders*

Larval ringed salamanders were collected from Kirby's Pond in Stone County, Missouri, approximately once a week from 20 October 1994 to 11 May, 1995. Four 1m<sup>2</sup> quadrats were produced from PVC tubing and were set in place within the pond. Location of each quadrat within the pond at each sampling period was assigned by using randomly-generated numbers to determine the compass headings and distance from the perimeter of the pond; quadrats did not overlap. Specimens were collected by sweeping a large net through the quadrat in parallel rows for the entire area, followed by a second sweep of the quadrat with a smaller net in an 'S' formation.

Collections from each quadrat were sorted immediately on shore. During the first eight collection periods, the first 10 salamander larvae were euthanized and preserved by submersion in 70% EtOH; for subsequent collections only the first five salamander larvae sorted were kept and preserved. Larval ringed salamanders were measured with vernier calipers (to 0.1 mm) for SVL, MHW, and gape width (GW; gape width measured at posterior edges of the mouth).

Specimens were delivered to the University of Saskatchewan in August, 2012. Larval ringed salamanders were randomly selected from the overall collection and were dissected to analyze contents of the upper digestive tract to distinguish cannibals from non-cannibals. Ingested conspecifics found in the digestive tract of cannibals were not measured for morphometrics due to physical degradation from digestion and long-term preservation. However, hatchling salamanders with partially formed hind limbs collected from the same collection periods as cannibals were used to approximate the morphometrics and stable isotopic values of individuals representing potential prey for cannibals.

For the purpose of comparative morphology between cannibals and non-cannibals, the methods documented in Nyman et al. (1993) were followed wherever possible. Cannibals were strictly categorized as individuals that had ingested conspecifics that were clearly identifiable within their digestive tract. Similarly, non-cannibals were categorized as individuals with conspecifics absent from their stomach contents. Non-cannibal specimens selected for comparison with cannibals were restricted to those collected over the same time period as cannibalistic specimens (weeks 3-16) and having a minimum SVL no smaller than that of the smallest cannibal (16.3 mm) (Nyman et al., 1993). Due to the physical degradation of ingested conspecifics occurring as a result of partial digestion, hatchling salamanders collected from weeks 1–2 were used to provide approximate morphometrics and stable isotope values of consumed conspecifics.

Precipitation and temperature data for the collection area (Galena, Missouri) for the October to May collection periods for both 1983-84 and 1994-95 were obtained from the High Plains Regional Climate Center, Lincoln, Nebraska.

Sub-sampling from original collections of both salamanders species were performed using random number sets generated in Excel (Microsoft Corporation, Santa Rosa, CA, USA).

### 5.3.2 Stable isotope analysis

Specimens were freeze dried in a Labconco Corp. Freezone® freeze drier for approximately 24 hr. Freeze-dried whole body tadpoles were pulverized to a fine powder, weighed and packaged at the National Hydrology Research Center (NHRC) of Environment Canada, Saskatoon, SK, Canada. Dry powder samples were packaged in ~0.1 mg portions, using Elemental Microanalysis Ltd. 5 x 3.5 mm tin capsules. Samples were subsequently submitted for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  analysis to the Stable Isotope Hydrology and Ecology Research Laboratory at NHRC, and the Stable Isotope Laboratory of the Department of Soil Science, University of Saskatchewan. Values for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  were expressed relative to Vienna Pee Dee Belemnite (VPDB) and air, respectively in parts per thousand (‰) (Biasatti, 2004). Measurement errors of  $\pm 0.3\text{‰}$  and  $\pm 0.2\text{‰}$  (for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively) were observed, using replicates of two internal laboratory standards (albumen and bowhead whale baleen).

### 5.3.3 Statistical Analysis

#### *Long-toed Salamanders*

A principle component analysis was performed on SVL, MHW, HL, and PREHW, and the first two components were retained. These components were then analyzed using two 2-sample independent *t*-tests to identify morphological differences between larval populations between 2007 and 2008.

I used two approaches to validate my findings. First, I classified the cannibal status of the individuals based on head morphology and tested for difference in size and stable isotope signatures. Since collections were conducted over four separate sampling periods (June 2007, August 2007, July 2008, August 2008), analysis of larvae was initially conducted

independently for each sampling period. Morphometric data were log transformed where they violated parametric assumptions. Two group K-means cluster analyses were used to initially classify salamanders into two groups based on values of head morphology traits (HL, MHW, and PREHW). Larvae classified into the group with the larger head morphology were considered putative cannibal morphs while the groups of larvae exhibiting the smaller head morphology were considered putative typical morphs. Separate one-way multivariate analyses of variance (MANOVA) were used to identify differences in log-transformed values of mass and SVL, and in non-transformed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between groups of salamander larvae in each collection period.

In the second approach, larvae were re-classified into two groups using K-means cluster analyses based on  $\delta^{15}\text{N}$  values. Where salamander larvae were classified in this manner, the groups exhibiting the higher  $\delta^{15}\text{N}$  values were labelled the cannibal morphs and the group with lower values was labelled as the putative typical morphs. Differences in head shape were also assessed using a discriminate analysis with Wilk's lambda distribution analysis of allometric transformations of head morphometrics (HL, MHW, and PREHW). I performed an allometric transformation procedure from Reist (1985) as used by Nyman et al. (1993) to isolate shape components of head dimensions. The predicted variable (Y) was derived for each head morphometric for each individual from the formula  $Y = 10^k$ ; where k is the log adjusted value of e, and where  $e = \log Y - B(\log X - \log X_{\text{SVL}})$ ; where Y is the original head morphometric, B is the regression coefficient of log Y and log SVL, and  $X_{\text{SVL}}$  is the grand mean of SVL for all larvae. This transformation adjusts the original measurements to values expected for mean body size (Nyman et al., 1993).

Differences in temperature and precipitation for each collection year were compared using a one-way MANOVA.

### *Ringed Salamanders*

Larval salamanders were classified as cannibals or non-cannibals based on the presence or absence of conspecifics in their digestive tract, respectively. One-way analyses of variance (ANOVA) tests with post-hoc Tukey HSD pairwise comparisons were used to assess differences in log-transformed values of SVL, mass, MHW, and GW among cannibals, non-cannibals and hatchlings. A one-way MANOVA with post-hoc pairwise comparisons was used to identify differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among cannibal, non-cannibal, and hatchling salamanders. Log-transformed values of head morphometrics (MHW and GW) of larvae were tested between groups using two analyses of covariance (ANCOVA) with the log-transformed values of SVL as the covariate.

Head morphometrics were transformed using the aforementioned allometric transformation. A discriminate analysis with Wilk's lambda distribution test was used on the transformed head morphometrics to explore the relationship between head morphology and observed cannibalism.

Precipitation and temperature data collected from September of the previous year to June of the sampling year. Temperature and precipitation values were tested using a one-way MANOVA. Pond width was recorded at every collection period in 1994-95, however, such information was absent from the 1983-84 collection period described by Nyman et al. (1993). To identify the potential difference in pond condition between sampling years I used a predictive regression of precipitation, temperature, and pond widths from 1994-95 to produce representative widths for the pond in 1983-84.

Analyses in both studies were performed using Systat (Wilkinson, 1998). Outliers were removed where identified through the statistical software. A conservative testing procedure was adopted independently for each experiment by adjusting significance levels using the Holm-Bonferroni correction (Marcus et al., 1976), to reduce the risk of committing

a type one error (Sokal and Rohlf, 1995). All figures were produced using SigmaPlot (Systat Software, San Jose, CA, USA).

## 5.4 Results

### 5.4.1 Long-toed Salamanders

A total of 98 larvae (June 2007 n =24; August 2007 n =14; July 2008 n =21; August 2008 n =39) were sub-sampled from the 197 larvae collected between 2007 and 2008. Results of the PCA of the two sampling years indicated that the first two components described 98.5% of the total variation among variables (Table 5.1). The first principal component (PC1) described the vast majority of the total variance (97.3%) and described variance in overall size. The second principal component (PC2) described 1.2% of the total variance and characterized head shape. This component described variation in PREHW. The highly negative value of the eigenvector loading indicates that increasing values of PC2 corresponds to a decreasing PREHW. No significant difference was observed in the overall sizes between salamander larvae collected in 2007 and those collected in 2008, as characterized through a *t*-test of PC1. However, a subsequent *t*-test identified a significant difference in PC2 ( $t_{[89]} = -5.6$ ;  $P < 0.001$ ).

**Table 5.1:** Eigenvectors for each morphometric in each principle component and percent of total variation explained by each principle component. Bold values denote important variable contributions to principle components.

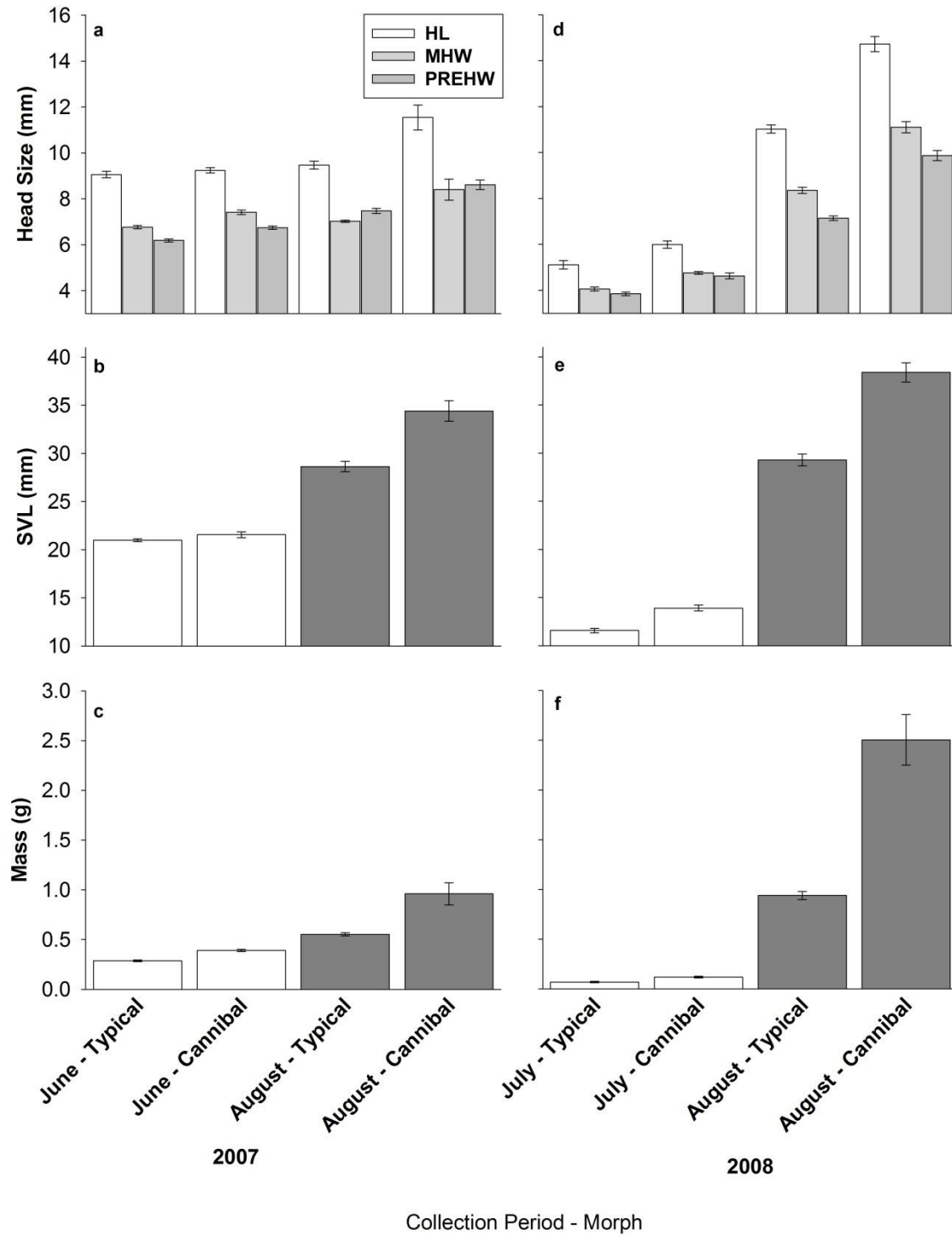
Variable	Eigenvectors	
	PC1	PC2
Mass	<b>0.449</b>	0.257
HL	<b>0.449</b>	0.313
MHW	<b>0.448</b>	0.465
SVL	<b>0.446</b>	-0.328
PREHW	<b>0.444</b>	<b>-0.716</b>
Percent of total variation	97.4	1.2



Multivariate analysis of salamanders collected in June 2007 clustered based on head morphometrics (Fig. 5.1a) exhibited significant overall differences in SVL, mass,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Hotelling-Lawley trace:  $F_{[4,19]} = 12.2$ ;  $P < 0.001$ ). Univariate  $F$ -tests indicated that larvae classified in the cannibal group exhibited significantly larger SVL ( $F_{[1,22]} = 7.2$ ;  $P = 0.01$ ), mass ( $F_{[1,22]} = 56.1$ ;  $P < 0.001$ ; Fig. 5.1b-c), and  $\delta^{15}\text{N}$  ( $F_{[1,22]} = 5.9$ ;  $P < 0.05$ ; values summarized in Table 5.2). However, no significant difference was observed in  $\delta^{13}\text{C}$  values between cannibal and typical groups of salamanders (Table 5. 2; Fig. 5.2). When these salamanders were re-clustered based on  $\delta^{15}\text{N}$  values, multivariate analysis identified a significant difference between the putative cannibal and typical groups (Wilk's Lambda = 0.5;  $F_{[3,13]} = 7.3$ ;  $P < 0.05$ ). Subsequently, discriminant analysis of the transformed head morphometrics correctly classified 100% of typical morphs (13 of 13) and 50% of cannibal morphs (2 of 4) with a total correct classification of 88%.

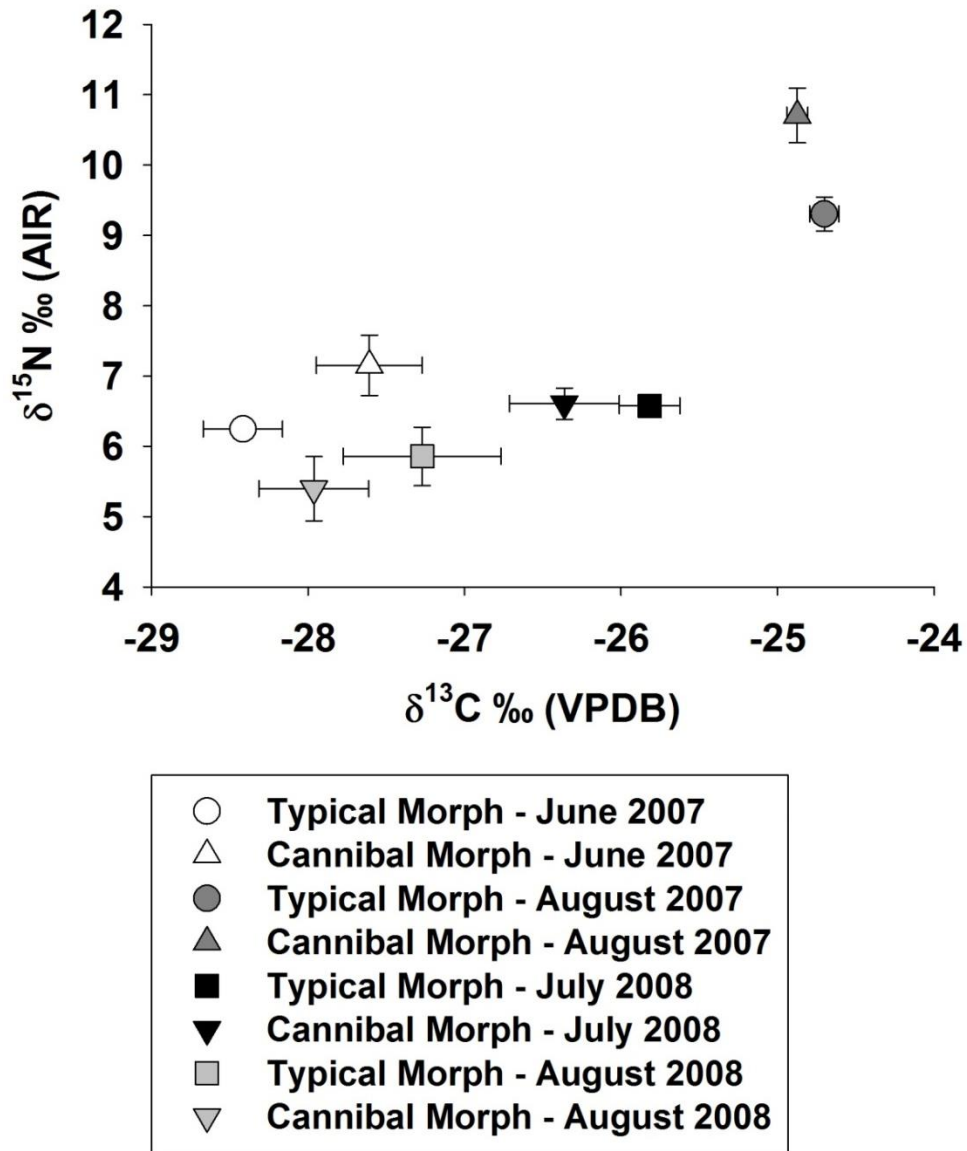
**Table 5.2:** Mean ( $\pm$  SE) values of mass, snout-vent length (SVL),  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  for larval long-toed salamanders clustered into putative cannibal or typical morphology groups based on head morphometrics collected in June and August 2007, and July and August 2008. Symbols denote significant differences between putative cannibal and typical groups for each sampling period: †  $\leq 0.001$ ; ‡  $\leq 0.01$ ; \*  $\leq 0.05$ .

Year	Month	Morphology	n	Mass (g)	SVL (mm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
2007	June	Cannibal	4	$0.4 \pm 0.0$	$21.5 \pm 0.3$	$-27.6 \pm 0.3$	$7.2 \pm 0.4$
		Typical	12	$0.3 \pm 0.0$ ‡	$20.9 \pm 0.1$ ‡	$-28.4 \pm 0.2$	$6.2 \pm 0.1$ *
	August	Cannibal	3	$0.9 \pm 0.1$	$34.4 \pm 1.1$	$-24.7 \pm 0.1$	$10.7 \pm 0.4$
		Typical	11	$0.6 \pm 0.0$ †	$28.6 \pm 0.6$ †	$-24.9 \pm 0.1$	$9.3 \pm 0.2$ *
2008	July	Cannibal	12	$0.1 \pm 0.0$	$13.9 \pm 0.3$	$-27.9 \pm 0.4$	$5.4 \pm 0.4$
		Typical	9	$0.1 \pm 0.0$ †	$11.6 \pm 0.2$ †	$-27.3 \pm 0.5$	$5.8 \pm 0.4$
	August	Cannibal	14	$2.5 \pm 0.3$	$38.4 \pm 1.0$	$-26.4 \pm 0.4$	$6.6 \pm 0.2$
		Typical	29	$0.9 \pm 0.0$ †	$29.2 \pm 0.6$ †	$-25.8 \pm 0.2$	$6.6 \pm 0.1$



**Figure 5.1:** Comparison of mean ( $\pm$  SE) head length, maximum head width, and pre-ocular head width (a), snout-vent length (b), and mass (c) among putative typical and cannibal morphs of larval long-toed salamanders collected in June and August 2007.

Multivariate analysis of salamanders collected in August 2007 identified overall differences between individuals grouped based on head morphometrics, where predicted PREHW values were included (Figure 5.1a) (Hotelling-Lawley trace:  $F_{[4,9]}=12.2$ ;  $P=0.001$ ). Univariate  $F$ -tests subsequently identified significant differences in mean SVL ( $F_{[1,12]}=20.2$ ;  $P=0.001$ ), mass ( $F_{[1,12]}=46.6$ ;  $P<0.001$ ; Fig. 5.1b-c), and  $\delta^{15}\text{N}$  values ( $F_{[1,12]}=46.6$ ;  $P<0.05$ ; values summarized in Table 5.2). No significant difference was observed in mean ( $\pm\text{SE}$ ) values of  $\delta^{13}\text{C}$  between groups (Table 5.2; Fig. 5.2). When re-classified based on  $\delta^{15}\text{N}$  values, multivariate analysis identified significant differences between groups in transformed head morphometrics (Wilk's Lambda = 0.4;  $F_{[3,10]}=4.7$ ;  $P<0.05$ ). Discriminate analysis correctly classified a total of 93% of salamanders with 100% of the typical morphs being correctly identified (7 of 7) and 86% of cannibal morphs were correctly classified (6 of 7). Where predicted values of PREHW were excluded from analyses, an overall difference between groups (classified based on head morphology, excluding PREHW) was still identified using multivariate analysis (Hotelling-Lawley:  $F_{[4,9]}=14.2$ ;  $P=0.001$ ). Univariate  $F$ -tests also observed significant differences in SVL ( $F_{[1,12]}=15.7$ ;  $P<0.005$ ) and mass ( $F_{[1,12]}=47.6$ ;  $P<0.001$ ), however, no significant differences were observed between groups in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. No significant differences were observed between groups (classified based on  $\delta^{15}\text{N}$  values) using multivariate analysis of transformed head morphometrics where PREHW values were excluded.



**Figure 5.2:** Comparison of mean ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among putative typical and cannibal morph long-toed salamander larvae from all four collection periods in 2007 and 2008. Only salamanders from August 2007 exhibit relative positioning suggesting that putative cannibal morphs were consuming conspecifics ( $\delta^{13}\text{C}$  values similar to typical morphs, but with significantly higher  $\delta^{15}\text{N}$  values).

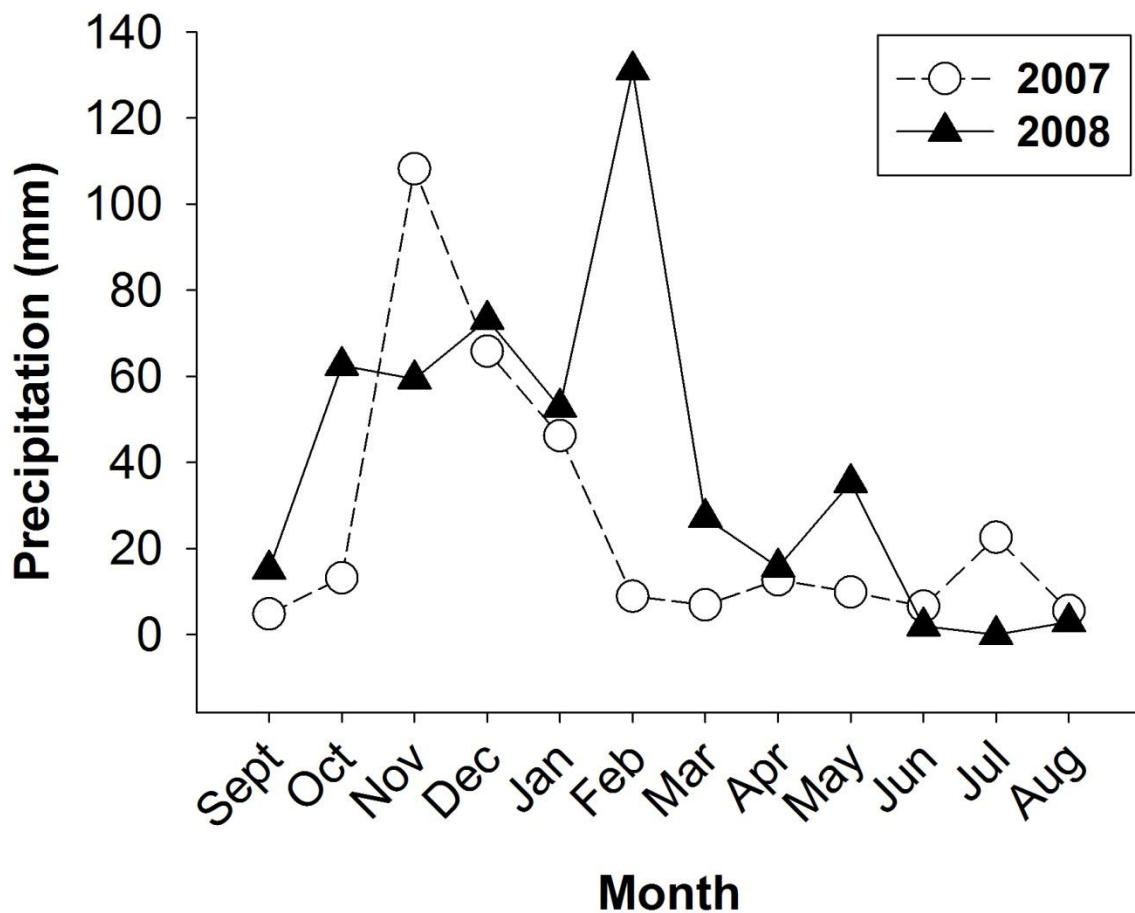
Significant differences were observed between salamanders grouped based on head morphometrics using multivariate analyses in both July (Hotelling-Lawley trace:  $F_{[4,16]} = 8.0$ ;  $P = 0.001$ ) and August (Fig. 5.1d) (Hotelling-Lawley trace:  $F_{[4,34]} = 29.1$ ;  $P < 0.001$ ) 2008. Univariate  $F$ -tests identified significant differences between cannibal and typical morph

groups in SVL (July:  $F_{[1,19]}=34.4$ ;  $P<0.001$ ; August:  $F_{[1,37]}=66.4$ ;  $P<0.001$ ; Fig. 5.1e) and mass (July:  $F_{[1,19]}=24.2$ ;  $P<0.001$ ; August:  $F_{[1,37]}=121.1$ ;  $P<0.001$ ; values summarized in Table 5.2; Fig. 5.1f). However, no significant differences were observed in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  (values summarized in Table 5.2; Fig. 5.2) in either month. When salamander larvae were reclassified into groups based on  $\delta^{15}\text{N}$  values no significant differences were observed through multivariate analyses between cannibal and typical groups in either July or August. Multivariate analyses indicated no significant differences between salamander groups in overall head morphometrics in July or August.

Multivariate comparison of weather data identified no overall significant difference between years. However, while not statistically different, the mean temperature was relatively lower in 2008 relative to that of 2007). Conversely, the mean ( $\pm\text{SE}$ ) precipitation was lower in 2007 than in 2008 (and the sum of precipitation was more than 150 mm lower in 2007 relative to the same time period in 2008 (values summarized in Table 5.3). Additionally, there was a greater influx of precipitation in early spring in 2008, which may have maintained or increased pond size during early larval salamander development (Fig. 5.3).

**Table 5.3:** Mean ( $\pm$  SE) temperature and precipitation, and total precipitation data for Sisters, OR between September 2006 – August 2007 and September 2007 – August 2008, and for Reeds Spring, MO between October 1983 – May 1984, and October 1994 – May 1995.

Location	Year	Mean Temperature ( $^{\circ}\text{C}$ )	Mean Precipitation (mm)	Total Precipitation (mm)
Oregon	2007	$8.1 \pm 2.1$	$25.9 \pm 9.2$	311.4
	2008	$7.2 \pm 2.1$	$39.8 \pm 11.1$	477.4
Missouri	1983	$7.6 \pm 2.7$	$104.1 \pm 15.8$	832.6
	1994	$9.2 \pm 1.9$	$115.9 \pm 26.6$	927.4



**Figure 5.3:** Monthly mean ( $\pm$  SE) precipitation data for Sisters, OR between September 2006 – August 2007, and September 2007 – August 2008.

#### 5.4.2 Ringed Salamanders

Of the 669 salamander larvae collected 124 larvae were sub-sampled for stable isotope and statistical analysis. A total of 14 salamander larvae were identified as definitively cannibals based on gut contents from the total collection and were only observed between collection weeks 3-16 (November 2, 1994 – February 7, 1995). A total of 73 non-cannibals collected from the same time period as the observed cannibals were randomly selected from the total collection. A total of 37 hatchling salamanders were collected from weeks 1 and 2

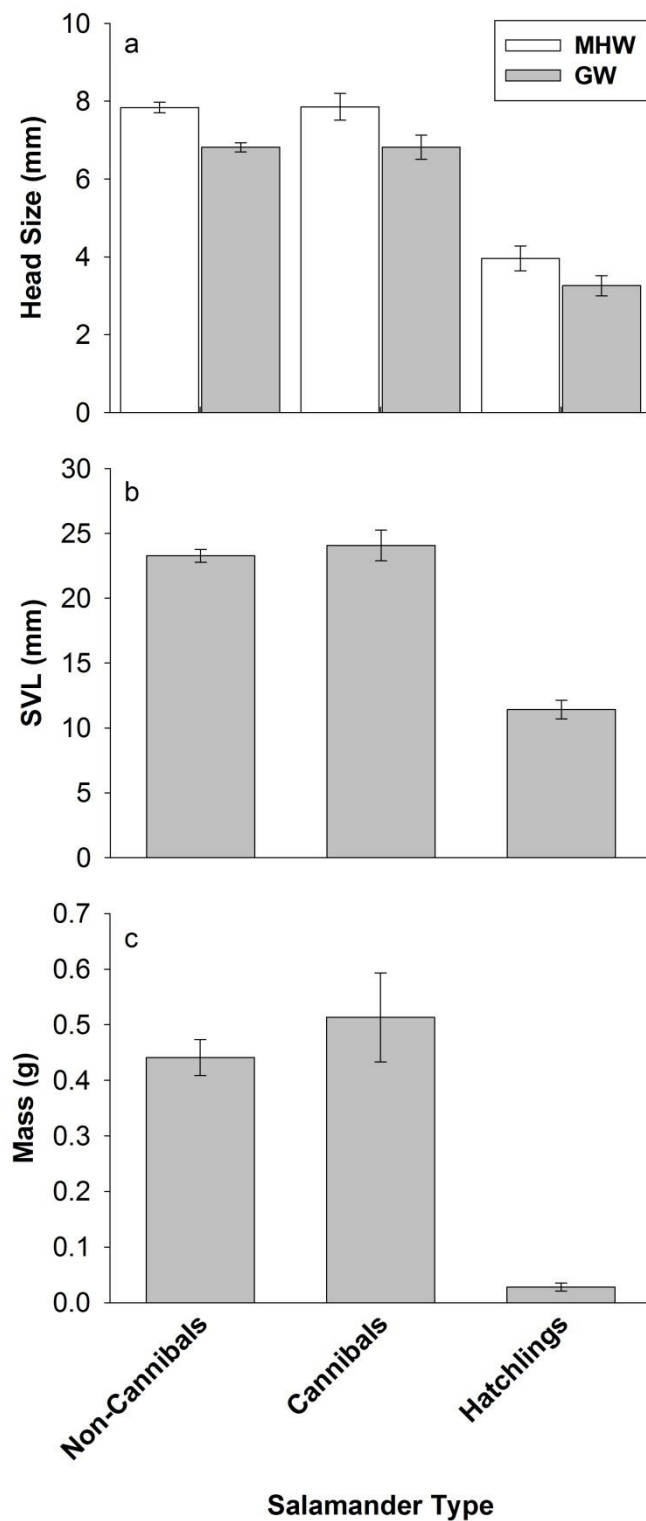
(October 20 – 28, 1994) and were used as approximations for putative conspecific prey in analyses of morphometrics and stable isotope values.

Among the three groups of salamanders, overall significant differences were observed in SVL ( $F_{[2,123]} = 209.2$ ;  $P < 0.001$ ), mass ( $F_{[2,123]} = 137.4$ ;  $P < 0.001$ ), MHW ( $F_{[2, 123]} = 165.4$ ;  $P < 0.001$ ), and GW ( $F_{[2, 123]} = 167.9$ ;  $P < 0.001$ ). Pairwise comparisons identified no difference between cannibals and non-cannibals in SVL, mass, MHW or GW (all  $P > 0.05$ ; values summarized in Table 5.4). Hatchlings were significantly smaller in SVL, mass, MHW, and GW relative to both cannibals and non-cannibals (all  $P < 0.001$ ; summarized in Table 5.4; Fig. 5.4). These results further validate that hatchlings represent potential prey as their mean MHW (the widest portion of their body) of hatchlings is smaller than the mean gape width of both cannibals and non-cannibals (Fig. 5.4a).

**Table 5.4:** Mean ( $\pm$  SE) values of mass, snout-vent length (SVL), maximum head width (MHW), gape width (GW),  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  among cannibalistic, non-cannibalistic, and hatchling larval ringed salamanders. Different letters denotes significant difference between groups.

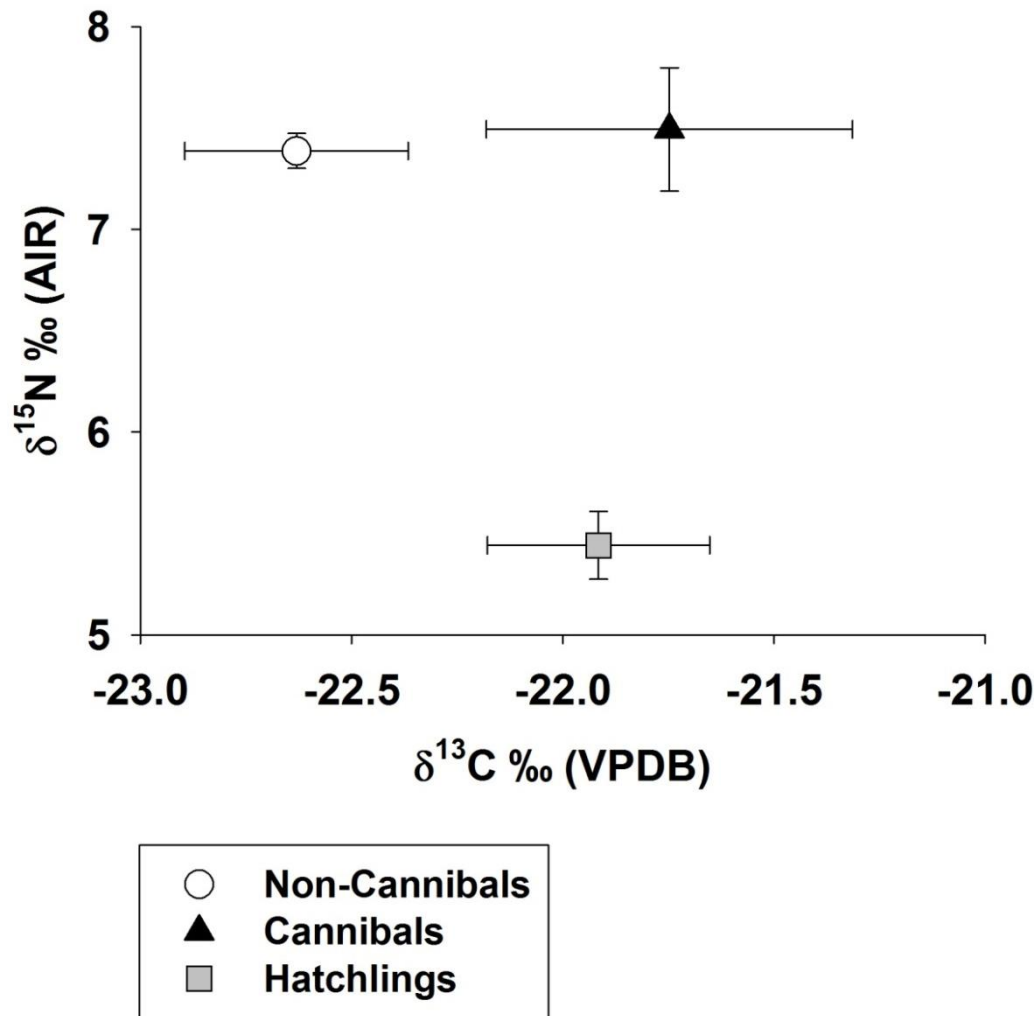
Group	n	Mass (g)	SVL (mm)	MHW (mm)	GW (mm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Cannibal	15	$0.5 \pm 0.1^a$	$24.1 \pm 1.2^a$	$7.8 \pm 0.3^a$	$6.8 \pm 0.3^a$	$-21.7 \pm 0.4^a$	$7.5 \pm 0.3^a$
Non-Cannibal	73	$0.4 \pm 0.0^a$	$23.3 \pm 0.5^a$	$7.8 \pm 0.1^a$	$6.8 \pm 0.1^a$	$-22.6 \pm 0.3^a$	$7.4 \pm 0.1^a$
Hatchling	38	$0.03 \pm 0.01^b$	$11.4 \pm 0.7^b$	$3.5 \pm 0.2^b$	$2.9 \pm 0.2^b$	$-21.9 \pm 0.3^b$	$5.4 \pm 0.2^b$





**Figure 5.4:** Mean ( $\pm$  SE) morphometrics of cannibal, non-cannibal, and hatchling larval ringed salamanders. Maximum head width, and gape width (**a**), snout-vent length (**b**), and mass (**c**) among cannibals, non-cannibals, and hatchlings of larval ringed salamanders collected between October 1994 – February 1995.

Multivariate analysis of stable isotopic values of the three salamander groups identified an overall significant difference among these groups (Hotelling-Lawley trace:  $F_{[4, 242]} = 33.1$ ;  $P < 0.001$ ). Univariate  $F$ -tests identified no significant differences among groups in  $\delta^{13}\text{C}$  values, however, an overall significant difference was observed among groups in  $\delta^{15}\text{N}$  values ( $F_{[2, 123]} = 64.7$ ;  $P < 0.001$ ; Table 5. 4). Pairwise comparisons identified significant differences in  $\delta^{15}\text{N}$  values between hatchlings with both cannibals and non-cannibals (both  $P < 0.001$ ; Table 5.4; Fig. 5.5), however, no significant difference was observed between cannibals and non-cannibals.

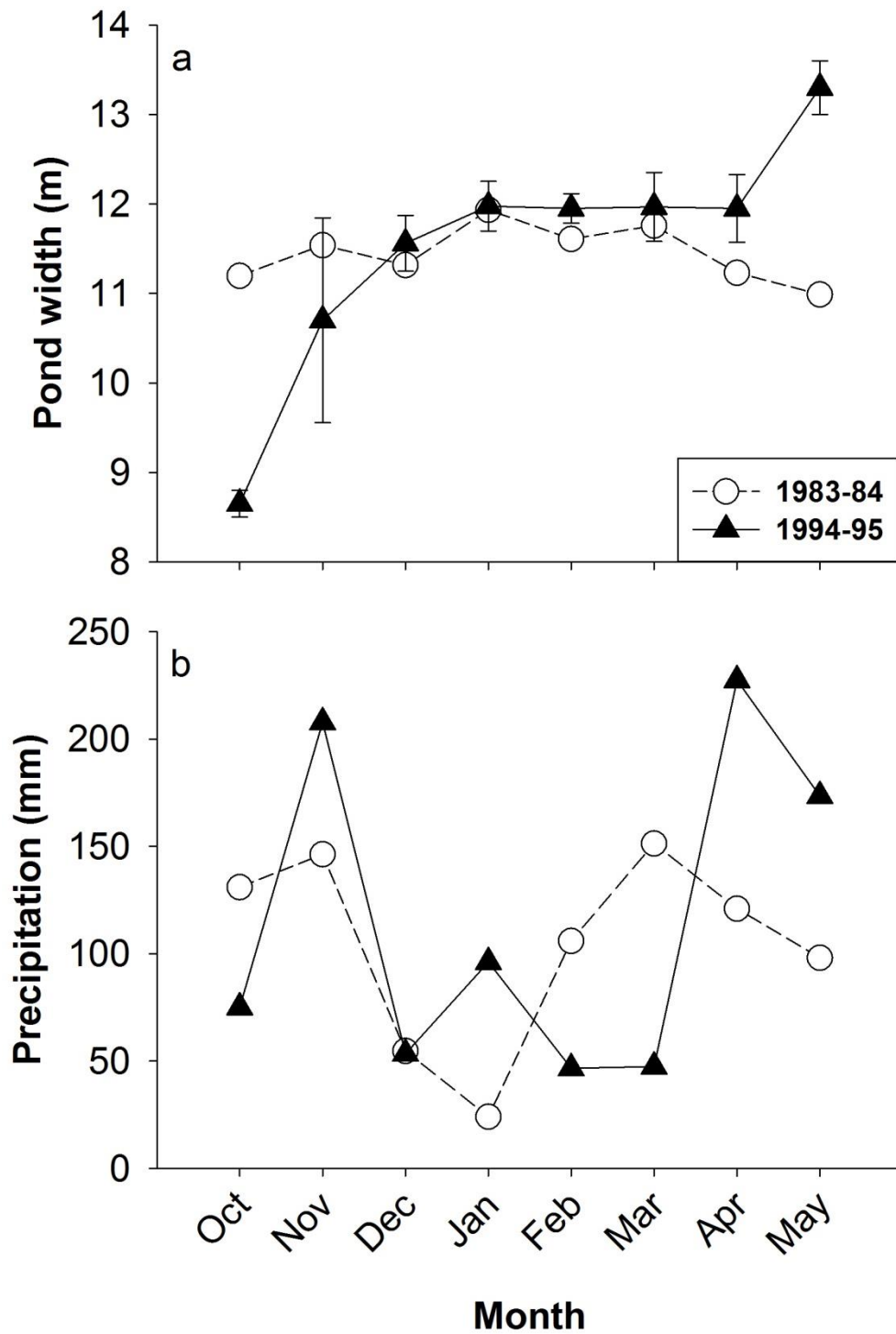


**Figure 5.5:** Mean ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among cannibal, non-cannibal, and hatchling ringed salamander larvae.

Comparison of head morphology between cannibal and non-cannibal larvae identified no difference in the ratios of MHW or GW to SVL (equivalent slopes). No differences in either head morphometric were observed when adjusted for differences in SVL.

No significant difference in overall weather was observed between years through multivariate analysis. However, mean ( $\pm$  SE) precipitation and temperature was higher in 1994-95 relative to 1983-84. Similarly, the total observed precipitation was nearly 100 mm greater in 1994-95 relative to 1983-84 (results summarized in Table 5.3). Predictive regression suggested that pond width potentially remained relatively consistent or shrank

slightly from October 1983 to May 1984, based on weather data from this period. Nyman et al. (1993) observed that the pond had a maximum surface area of  $150 \text{ m}^2$  and a depth of  $\leq 70$  cm and this was reduced through the summer. Conversely, pond measurements recorded over the 1994-1995 collection period show an increase in pond width by approximately 5m from October 1994 (mean  $\pm$  SE:  $8.6 \pm 1.5$  m) to May 1995 (mean  $\pm$  SE:  $13.3 \pm 0.3$  m; Fig. 5.6a). Additionally, I observed relatively high precipitation in November 1994, which corresponds with the initial increase in pond size. Similarly, the additional influx of precipitation during the colder winter months and early spring appear to maintain and increase pond size throughout the developmental period of these larvae (Fig. 5.6a,b).



**Figure 5.6:** Mean ( $\pm$  SE) pond widths of Kirby's pond observed from October 1994 – May 1995 and the predicted pattern of pond width over the same time period in 1983-84 (**a**), and the monthly mean precipitation observed in the area for both time periods (**b**).

## 5.5 Discussion

The results of this study suggest that a linkage between cannibalism and head morphology occurs within natural populations of both species of larval salamanders. However, the necessity for enlarged head morphology to facilitate cannibalism appears related to the breeding strategies of salamanders. Additionally, my results suggest the expression of polymorphisms within these larval populations is transient and potentially related to the hydrological condition of natal ponds.

Food availability and conspecific density influence the expression of intraspecific aggression and cannibalistic behaviour (Collins and Cheek, 1983; Griffiths, 1997; Hoffman and Pfennig, 1999; Pedersen, 1993; Wildy et al., 2001). Where larval salamander densities are naturally high, and/or where pond conditions act to increase larval densities (i.e. pond drying), larvae may be forced into aggregations around limited food resources, leading to increasingly high degrees of competition (Griffiths, 1997; Wells, 2007). Peacor and Pfister (2006) indicated that intra-population size variation of larval amphibians raised at high population densities resulted from phenotypic adaptations ('size-independent' factors), causing differences in foraging efficiency among individuals (Fuiman and Cowan, 2003). Acquisition of limited resources can therefore be improved in individuals with these advantageous traits (Peacor and Pfister, 2006). The effects of these traits then become increasingly pronounced with increasing competition, and thus an increased size disparity develops among individuals within the population (Peacor and Pfister, 2006). Therefore, increasing density could potentially result in the expression of a specialized head morphology that facilitates cannibalism. Simultaneously high larval density could provide the opportunity for cannibalism by forcing putative cannibals and their prey into proximity of each other.

My results suggest larval long-toed salamanders exhibited variation in their head morphologies in 2007 (Figure 5.1a), and those individuals exhibiting the larger head

morphology occupied a significantly higher trophic position based on  $\delta^{15}\text{N}$  values. Additionally, the observation that putative cannibals exhibited a significantly greater mass than typical larvae was similarly observed by Wildy et al. (1998). These results suggest the presence of a linkage between cannibalism and head morphology. However, no such relationship was observed among larval salamanders collected from the same wetland in 2008. This difference may be due to the concurrent breeding strategy of long-toed salamanders (Petranka, 1998). Since these salamanders breed explosively, all larvae within a population should be approximately the same age and of similar size. Petranka and Thomas (1995) suggest that the evolution of synchronous breeding was influenced by the efficiency of larval amphibian cannibalization of vulnerable conspecifics. Indeed, synchronized breeding can reduce the risk of cannibalism by limiting the differences in size and development among individuals (Crossland et al., 2011; Petranka, and Thomas, 1995). Therefore, specific adaptations in head morphology (i.e. the cannibal morphology) may greatly facilitate the ability of individuals of this species to consume conspecifics (Michimae and Wakahara, 2001; Pierce et al., 1983; Reilly et al., 1992; Rose and Armentrout, 1976). The greater gape size of the putative cannibal morphs provides these individuals an initial benefit by improving their ability to consume larger prey, including similarly sized conspecifics (Pierce et al., 1983). Successful cannibals may experience subsequent predation success resulting from the increased growth facilitated by cannibalism (Lannoo, and Bachmann, 1984; Rose and Armentrout, 1976; Wildy et al., 1998).

My observations of larval ringed salamanders appear to contradict those of Nyman et al. (1993) who found significant differences in head morphology and shape between cannibals and non-cannibals. Additionally, Nyman et al. (1993) observed the presence of cannibalistic larvae within the pond until April, approximately to the observed initiation of metamorphosis. Conversely, my last observation of cannibalism occurred in February.

Ringed salamanders breed over a period of a month or more, providing a natural size differentiation among larvae of different age classes (Petranka, 1998). This moderately extended breeding period may allow cannibalism to occur without the expression of specific head morphologies (Nyman et al., 1993; Petranka, 1998). However, it is possible the expression of larger head morphology could prolong the period during which larvae can consume conspecifics. Alternatively, if larval densities were sufficiently low the occurrence of cannibalism may also be reduced due to insufficient opportunity (Wells, 2007). Similarity in stable isotope values between cannibal and non-cannibal groups could be the result of one or more of the following situations: 1) gut content analysis provides only a snap-shot of feeding; cannibalism may have occurred but was not observed in members of the non-cannibal group because conspecific prey were completely digested prior to capture; 2) cannibalistic individuals were observed to have ingested conspecifics but had not digested this prey and therefore would not have assimilated this diet into their tissues; and 3) cannibalism does not represent a significant contribution to the overall diet of salamanders and therefore does not significantly alter the isotopic values of cannibals (McCarthy et al., 2004). The most probable cause of this result is that cannibalism represents a relatively low proportion of the larval salamander diet. Conspecific prey was sufficiently large to fill and/or exceed the upper digestive tract of the observed cannibals. This suggests that digestion would take much longer than smaller invertebrates, which make up the majority of larval salamander diet (Nyman et al., 1993). Therefore, it is possible that cannibalism is relatively common among individuals, however, the frequency of cannibalistic behaviour in any individual would be relatively low.

The transient nature of these trophic polymorphisms may have been, in part, related to the differences in precipitation between collection years. Brooks (2004) observed that the water level of temporary wetlands was significantly related to precipitation. I observed that



while there was no significant difference in precipitation or temperature between collection years in either study, the sum of precipitation was greater in years where cannibalistic morphs were absent. More importantly, the timing of the observed influxes of precipitation suggests that pond size may have been maintained or increased in cases where no differentiation in head morphology was observed among larval salamanders. My results appear consistent with those that may be expected. However, the absence of comprehensive data regarding larval densities, prey abundance, and pond conditions during all sampling periods means it is not possible to definitively conclude this timing as evidence of a causative relationship.

The results of this study support the linkage between morphology and cannibalism in larval salamanders within natural populations and the density-dependent nature of this relationship. The expression of rapid phenotypic adaptations to facilitate cannibalism may be the result of increasing competition [Blaustein et al., 2001; Jefferson et al., 2013 (Chapter 3); Jefferson et al., 2014ab (Chapters 4 and 2); Wildy et al., 2001]. Differences in life history among salamander species and weather patterns may also have important consequences for the expression of polymorphisms and cannibalism. The ubiquitous assignment of polymorphism across larval salamander species that exhibit cannibalism may therefore be inaccurate.

If these assumptions are correct there may be serious implications to population dynamics and survivorship of larvae that could result from changes in climate and/or habitat (Brooks, 2004). Long-term monitoring of polymorphic larvae in natural populations, and comparison across multiple species may therefore be necessary to develop a more complete understanding of the dynamics of this phenomenon.

Trophic polymorphisms are a potential competitive adaptation expressed by larval salamanders under conditions of high competition. Concurrent breeding species, such as long-toed salamanders may be dependent upon such polymorphisms to facilitate intraspecific

predation. Species that are not concurrent breeders may be cannibalistic without exhibiting the polymorphisms due to size differentiation inherent within the population; however, individuals exhibiting the cannibal morphology may be capable of intraspecific predation for longer periods of time. Additionally, it appears as though the expression of these trophic polymorphisms may be influenced by seasonal precipitation and temperatures. Therefore, trends towards warmer drier climates could influence population dynamics of larval salamanders thereby resulting in increased expression of trophic polymorphisms and cannibalistic behaviour.

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## **Chapter 6: General Discussion**

### **6.1 The Conditional Benefits of Cannibalism**

Conspecific tissues represent a theoretically ideal diet, yet the results of my research suggest these benefits are not necessarily realized in practice [Jefferson et al., 2014b (Chapter 2); Meffe and Crump, 1987]. Mine is not the first study to observe a diet of conspecifics to yield less than expected benefits to cannibals [Jefferson et al., 2014b (Chapter 2)]. Pizzatto and Shine (2008) observed that once juvenile cane toads attain sufficient size to consume smaller conspecifics, cannibalism may represent up to 67% of the biomass of their prey. However, the authors also observed that while juveniles actively consume smaller conspecifics, such a diet yielded significantly inferior growth in toads relative to those fed an equivalent mass of ants (Pizzatto and Shine, 2008). Pizzatto and Shine (2008) note that these deleterious effects appear unrelated to toxins present in the skin of the consumed toads, yet the apparent disconnect between potential and realized nutritional benefits are “puzzling”.

It is possible that the apparent lack of benefits in the study by Pizzatto and Shine (2008) could be the result of innate differences in condition between the smaller and larger toads. The theory that cannibalism is an ideal diet is predicated on the notion that such a diet contains all of the necessary nutrients, in approximately the right proportions (Meffe and Crump, 1987). However, dramatic changes occur during metamorphosis including a complete alteration of the entire digestive tract, and feeding may cease in tadpoles prior to the initiation of metamorphosis (Gosner, 1960). Additionally, depending upon experiences of larvae the condition of newly metamorphosed individuals may be impacted resulting in smaller individuals (Audo et al., 1995; McCallum and Trauth, 2002). In other words, the lack of realized benefits to larger toads may have been due to smaller toads being of inferior



nutritional quality relative to the larger cannibals, and therefore lack the composition that would otherwise make their tissues an ideal diet.

My research matched the potential cannibals with prey of similar age and size. Consequently, the argument that tadpoles are unable to efficiently digest and/or assimilate animal prey appears unlikely due to the relative benefits they appeared to derive from the invertebrate diet provided. Conversely, the observed variation in size that was noted among tadpoles fed conspecific tissues relative to tadpoles fed other diets, suggests that individuals exhibited differentiation in their foraging behaviour when presented with conspecific tissues. Similarly, the preferential avoidance in consuming conspecific tissues observed in the initial tadpole feeding experiment, and the subsequent “catch-up growth” once tadpoles initiated feeding, suggests that the lack of benefits are not necessarily due to nutritional deficits but rather could be the result of behavioural inhibition. Here, individual wood frog tadpoles appear innately opposed to consuming conspecifics, yet may possibly derive great nutritional benefit when they do (Pizzatto and Shine, 2008). The lack of perceived benefits may therefore be the result of differences in willingness of individuals to consume conspecific tissues.

Wood frog tadpoles may be such generalized consumers that as long as tadpoles receive sufficient protein in their diet they may achieve relatively high rates of growth and development. This appears supported by the observation that wood frog tadpoles fed brine shrimp in Experiment 1 (Chapter 2; Fig. 2.1) did not exhibit greater growth or development relative to larvae collected from natural populations at points through the experiment. If brine shrimp was better than the dietary resources consumed in their natural environment, they would be expected to exhibit significantly greater growth and/or development. Schiesari et al. (2009) noted that wood frog tadpoles in closed canopy wetlands appear to function efficiently as primary predators, and are far more omnivorous in open canopy wetlands. This suggests

that they can efficiently alter their diets to adapt to the conditions of their habitat, therefore cannibalism, all else being equal, should not represent any less of a diet than brine shrimp. This again appears to suggest behavioural and/or personality differences among individuals.

Alternatively, the research presented in this thesis did not account for differences in carbohydrate and lipid composition, or caloric differences among diets. Given the similarity in protein content between brine shrimp and conspecific tissues it stands to reason that nutritional deficits may have resulted from lower carbohydrate and/or lipid content of conspecific tissues. The attempt was made to compensate for such discrepancies by gut loading tadpoles that were sacrificed to produce cannibalistic diets, however these benefits may have been insufficient. Future experiments should attempt to analyze carbohydrate and lipid composition, and conduct calorimetric analyses of conspecific tissues and alternate diets.

Conversely, Wildy et al. (1998) documented improved growth in cannibalistic long-toed salamanders. Cannibalistic individuals had a significant increase in mass when fed a diet of conspecifics relative to a diet of tadpoles, or a mixed diet of tadpoles and conspecifics. Similarly, studies have observed improved growth and in some cases accelerated time to metamorphosis in cannibalistic tiger salamander larvae, relative to non-cannibals (Lannoo et al., 1989; Lannoo and Bachmann, 1984).

Wood frog tadpoles are less predatory relative to ambystomatid salamander larvae, and certainly lack any specialized features for carnivory (Wells, 2007). It may then be reasonable to assume that the results observed in tadpoles are not applicable to salamander larvae because of a disparity in digestive efficiency of animal proteins between these two groups of larval amphibians. However, wood frog tadpoles appear to show favourable growth and development when provided a purely brine shrimp diet, and commonly consume animal tissues in nature, including those of other amphibians [Bleakney, 1958; Holbrook et al., 2004;

Jefferson et al., 2014b (Chapter 2); Petranka and Thomas, 1995; Petranka et al., 1998; Schiesari et al., 2009]. Additionally, conspecific tissues were also homogenized, providing a great deal of physical breakdown of the tissues prior to tadpoles ingesting them.

One point that may explain the difference in observed benefits between tadpoles and larval salamanders is that cannibalistic salamanders are often easier to identify than cannibalistic tadpoles. Larval salamanders are gape limited predators that typically consume their prey whole, while tadpoles must tear large prey apart with their keratinized jaw sheaths to consume them. This means gut content analysis will favour observation of cannibalism in larval salamanders over tadpoles. Additionally, cannibalistic salamanders are often far larger and may exhibit the cannibal morphology making them easier to identify, while cannibalistic wood frog tadpoles have not yet been observed to exhibit an obvious difference in appearance. In other words the observation that tadpoles do not exhibit ideal growth and development when provided conspecific tissues as a diet may be more to do with a difference among individuals (i.e. behavioural, personality, and/or physiological adaptations) than a general deficiency in the diet. Ideally, future research will focus on identifying what, if any, differences exist among individuals that may favour and/or facilitate cannibalism.

## **6.2 Nutritional Stress**

Risks associated with cannibalism should be disregarded when an animal is at risk of starvation, as certain death otherwise negates other risks to future fitness (Dawkins and Krebs, 1979). A study by Bleakney (1958) suggests cannibalism sustained a population of larval wood frogs where no alternate food was observed. Results of my research suggest that larvae concede to cannibalism when faced with starvation [Jefferson et al., 2014b (Chapter 2)]. Whole body corticosterone concentration was significantly elevated in starved tadpoles [Jefferson et al., 2014b (Chapter 2)]. Corticosterone stimulates tadpole appetite following

periods of anorexia in order to “maintain energetic homeostasis” (Crespi and Denver, 2004; Denver, 2009). Increased appetite induces increased foraging behaviour, and where necessary this could encourage cannibalistic behaviour (Crespi and Denver, 2004). Increased aggression has also been observed among larval salamanders in response to dietary stress, and may culminate in cannibalistic behaviour (Manenti et al., 2015).

Venesky et al. (2012) has suggested that poor quality diets may invoke a similar stress response. However, results of my research indicates that short term dependency upon a poor quality diet may be insufficient to provoke a significant hormonal response, suggesting that poor dietary quality alone will not necessarily invoke a cannibalistic response [Jefferson et al., 2014b (Chapter 2)]. When tadpoles were fed a poor quality diet they failed to exhibit a significant elevation in whole body corticosterone. This suggests that while low dietary quality may eventually stimulate cannibalistic behaviour, it may require prolonged reliance upon such a diet to enact a cannibalistic response (Audo et al., 1995; McCallum and Trauth, 2002). McCallum and Trauth (2002) found that tadpoles fed the same low protein diet as used in my study (cornmeal) exhibited much lower survivorship (14%) relative to tadpoles fed a high protein diet (100% soybean: 64%), and exhibit a far longer developmental period (cornmeal: 212 d; soybean: 133 d) [Jefferson et al., 2014b (Chapter 2)]. Therefore, despite the potential detrimental impacts of such poor quality diets, tadpoles may not innately resort to cannibalism based on dietary quality alone [Jefferson et al., 2014b (Chapter 2); McCallum and Trauth, 2002]. Additionally, results suggest that supplementing larval diet with conspecific tissues generally does not provide significant benefits [Jefferson et al., 2014b (Chapter 2)].

### 6.3 Perception of Risks

Release of chemical alarm cues are typically used to communicate the presence of a potential threat to conspecifics in the vicinity (Denver, 2009; Fraker et al., 2009; Ferrari et al., 2010). Tadpoles typically respond to this potential risk by rapidly inhibiting behaviour, settling on pond bottoms, and limiting activity to reduce detection by predators (Denver, 2009). Since cannibalism requires an individual to cause injury to a conspecific, chemical alarm cues should innately limit, if not deter cannibalism. The observation of individual tadpoles preferentially avoiding consuming conspecific tissues may have been influenced by these chemical cues. In the absence of an alternative food source tadpoles may have avoided conspecific tissues simply because they were also the emitting source of the chemical alarm cues, and thus may have appeared to be a risk with no perceived reward.

However, ecological conditions are rarely so simplistic, and larvae must express the most efficient response within the overall ecological context of the habitat (e.g. Michel, 2012; Relyea, 2004; Richter-Boix et al. 2007). Alarm cues appear to dissipate or deteriorate over a relatively short period of time in natural conditions, allowing individuals to return to previous activities and/or focus on alternative sources of information (e.g. competition, food, etc) (Ferrari, et al., 2008). Not all species respond similarly to risk. Dayton and Fitzgerald (2011) observed that tadpoles of Couch's spadefoot toads exhibit no defensive responses (e.g. behavioural, developmental, or morphological) to predator cues. These tadpoles develop in very short duration wetlands, and often must rapidly develop to metamorphosis (between 8-60 d) to prevent mortality from pond desiccation (Dayton and Fitzgerald, 2011; Newman, 1987). Investment in behavioural or morphological defences could undermine this rapid development (Dayton and Fitzgerald, 2011). Instead, predation naturally limits competition for depleting resources, allowing survivors to grow faster and accelerate time to metamorphosis. Wildy and Blaustein (2001) observed that larval long-toed salamanders lack

an innate avoidance of cannibalistic individuals using chemical cues; instead they required experience in order to learn to avoid these individuals. Additional cues released from injured conspecifics may also be perceived as rising population density suggesting an influx of competitors. Alternatively, results suggest there may also be some attractive cues released indicative of available prey, and therefore act as a dinner bell to entice additional larvae creating a feeding frenzy [Jefferson et al., 2014b (Chapter 2)].

#### **6.4 Opportunity and Priority Effects**

Often cannibalism is not the result of necessity but rather opportunistic exploitation of vulnerable prey. Many species of larval amphibians have been identified as consuming younger, more vulnerable conspecifics, and/or exhibiting morphological adaptations that may allow them to consume conspecifics (e.g., Collins and Cheek, 1983; Crump, 1983; Crump, 1990; Hawley, 2009; Jordan et al., 2004; McCallum and Trauth, 2001; Meffe and Crump, 1987; Michimae and Wakahara, 2001; Petranka and Thomas, 1995; Pizzatto and Shine, 2008; Nyman et al., 1993; Wildy et al. 2001; etc.). Where breeding asynchrony exists, offspring of early breeding individuals gain benefits from reduced risk of predation, adequate developmental period, and relative competitive advantages (Morin, 1987; Waldman, 1982). The offspring of the late breeding individuals however run the risk of predation from older conspecifics (Claessen et al., 2003; Petranka and Thomas, 1995; Polis, 1981). The efficiency by which some species may opportunistically consume vulnerable offspring has been suggested as a selective pressure towards synchronized breeding (Crossland et al., 2011; Petranka and Thomas, 1995). Breeding synchrony innately reduces the potential for such opportunity by greatly diminishing initial size variation among individuals within a population (Crossland et al., 2011; Petranaka and Thomas, 1995).

The results of my research suggest that breeding asynchrony can facilitate cannibalism within larval populations. The intensity of intraspecific predation, however, appears variable among species, and potentially variable among populations. My results suggest that such opportunistic cannibalism within populations of larval ringed salamanders may not be as intense as observed in wood frog tadpoles [Jefferson et al., 2014c (Chapter 5); Petranksa and Thomas, 1995]. Cannibalism was observed in only 11% of sampled larval ringed salamanders (14 out of 124), and in the absence of morphological adaptations this cannibalism appeared relatively limited within a shorter time frame [Jefferson et al., 2014c (Chapter 5); Nyman et al., 1993]. Results from observations of larval long-toed salamanders also confer with conclusions of Crossland et al. (2011) and Petranksa and Thomas (1995) suggesting that synchronous breeding will reduce the occurrence of cannibalism, and morphological adaptations to feeding structures appear necessary to facilitate cannibalism [Jefferson et al., 2014c (Chapter 5)].

## **6.5 Density, Competition, and Adaptive Responses**

Selection should result in adaptations that reduce predation, including cannibalism (Dawkins and Krebs, 1979; Lima and Dill, 1989; Ferrari et al., 2010). However, the underlying selective pressure of competition may contradict this by favouring heightened aggression and other adaptations that may facilitate cannibalism (i.e. polymorphism) (Collins and Cheek, 1983; Polis, 1981; Verner, 1977; Wildy et al., 2001). Where individuals aggregate around dietary resources individuals with the ability to be cannibalistic have the opportunity to do so, based on the presence of potential prey; therefore potential for cannibalism may increase with increasing density (Møller et al., 2008; Wells, 2007).

Results from my research suggest that larval wood frogs respond to cues indicative of increasing competition with increased propensity for consuming conspecific tissues

[Jefferson et al., 2013 (Chapter 3); Jefferson et al., 2014a,b (Chapters 4 and 2)]. This suggests that the risk of competition may invoke the expression of increasingly aggressive behaviour and/or bolder personalities in individual larvae. In other words, where larvae perceive a risk from high competition they may become more aggressive in feeding and/or show an increased willingness to consume a diet that itself may pose a perceived risk (i.e. alarm cues). Manenti et al. (2015) found that aggression among larval European fire salamanders (*Salamandra salamandra*) increased with density and dietary stress, and that this increased aggression increased the probability of intraspecific attacks and cannibalism. The authors suggest that the plasticity for aggression allows individuals to exist in areas of depleted resources (Manenti et al., 2015). Therefore, when larvae are forced into feeding aggregations individuals may become increasingly aggressive towards conspecific, and potentially exhibit agonistic behaviour possibly culminating in cannibalism.

Previous studies have suggested that expression of cannibalism and trophic polymorphism are density dependent (Collins and Cheek, 1983; Griffiths, 1997; Hoffman and Pfennig, 1999; Maret and Collins, 1997; Pedersen, 1993; Wildy et al., 2001). My results similarly suggest that larval salamanders may exhibited trophic polymorphisms in response to heightened population densities resulting from weather conditions facilitating rapid pond drying [Jefferson et al., 2014c (Chapter 5)]. Maret and Collins (1997) observed that dietary resource overlap between cannibal and typical morph larvae of the tiger salamander (*Ambystoma tigrinum nebulosum*) decreases with increasing larval densities. This indicates that advantages to individuals expressing the cannibalistic morphology are greatest when competition among typical morph larvae is at its highest (Maret and Collins, 1997). Svanbäck and Bolnick (2007) similarly observed that three-spine sticklebacks (*Gasterosteus aculeatus*) may exhibit different morphologies that facilitate the utilization of alternate prey, and that “competition also increased the diet–morphology correlations, so that the frequency-



dependent interactions were stronger in high competition”. The combination of benefits from the expression of trophic polymorphism, which may facilitate cannibalism, and opportunity afforded to putative cannibals with an abundance of potential prey (typical morphs) when population densities are high could result in cannibalistic behaviour (Crump, 1983; Fox, 1975a, b; Maret and Collins, 1997; Møller et al., 2008).

## **6.6 Environmental Influence**

Temporary wetlands are dynamic habitats with conditions that can dramatically fluctuate in response to those of the environment (Brooks, 2004; Griffiths, 1997; Maret and Collins, 1997; Reinhardt et al., 2015; Worthylake and Hovingh, 1989). Wetland size and hydroperiod is strongly influenced by environmental conditions (Brooks, 2004). Relatively low precipitation may result in decreased pond size, combined with high temperatures this could potentially lead to rapid pond drying [Brooks, 2004; Griffiths, 1997; Jefferson et al., 2014c (Chapter 5)]. The timing of influxes of water from precipitation may also be important, for example precipitation that leads to increased spring run-off and precipitation occurring during the developmental period of larvae may be more important than precipitation events at other times of the year [Jefferson et al., 2014c (Chapter 5); Maret and Collins, 1997]. The result of pond drying is reduced habitat size, which may in turn cause larval density to increase and a reduced developmental time (Griffiths, 1997). Increased population densities and/or reduced time to develop can result in increased competition among larvae, and thus potentially lead to increased aggression and agonistic behaviour and subsequently cannibalism (Collins and Cheek 1983; Wildy et al. 2001). Additionally, changes in temperature may alter prey abundance within ponds, and could alter larval feeding behaviour and potentially promoting cannibalism (Blaustein et al., 2010).

Results of my study of natural populations of larval ambystomatid salamanders suggest that the expression of trophic polymorphisms appear related to weather conditions that may reduce hydroperiod and/or size of wetlands [Jefferson et al., 2014c (Chapter 5); Nyman et al., 1993]. Reinhardt et al. (2015) also suggests differences in the expression of polymorphisms among larval European fire salamanders resulting from differences in weather conditions. Although both studies fundamentally argue that the expression of the polymorphisms is the result of resource limitation, they differ in the mechanistic pathway through which this occurs. Reinhardt et al. (2015) suggests that low temperatures result in trophic polymorphism by limiting the ability of larval salamanders to exploit profitable prey species while they co-exist.

Reinhardt et al. (2015) observed that low temperatures occurring during early development of larval salamanders resulted in trophic polymorphism in larval populations, and cannibalism was observed in the larger individuals. The authors suggest that the lower temperatures occurred at a time when larval salamanders and mosquito larvae (*Aedes vexans*) overlap in wetland occupation. The lower temperatures resulted in reduced ability of larval salamanders to exploit this highly profitable prey, which larval salamanders appear to be reliant upon for much of their growth (Reinhardt et al., 2015). Reinhardt et al. (2015) observed that the populations exhibited bimodal growth, with the majority exhibiting very limited growth, and a few individuals showing increasing growth. Individuals exhibiting increasing growth were also observed to have become cannibalistic and were the only group of larvae to survive to metamorphosis (Reinhardt et al., 2015).

Despite the difference in mechanism (low precipitation leading to rapidly shrinking wetlands vs. low temperature interfering with larval utilization of an important prey species) the results of my research and those of Reinhardt et al. (2015) are simpatico. Both studies implicate environmental conditions as important variables that may significantly influence

ecological conditions, alter population dynamics, and potentially influence the expression of adaptive responses including those that may ultimately lead to increased cannibalistic behaviour.

Weather patterns and long-term changes in climate can influence and potentially have dramatic impacts upon species through changes to habitat and community composition (Blaustein et al., 2010; Brooks, 2004; Petchey et al., 1999; Walther et al., 2002). As suggested in Jefferson et al. (2014c; Chapter 5) climate change could dramatically alter habitat size and population densities for larval salamanders potentially leading to increased instances of cannibalism as competition becomes increasingly intense. Climate change has also been suggested to influence the occurrence in cannibalism among American lobster (*Homarus americanus*). Growth in some north Atlantic populations of lobster is believed to be related to increased water temperatures and the decline of predatory ground fish through over fishing (Oppenheim and Wahle, 2003; Steneck and Wahle, 2013). The subsequent increase in population densities resulting from these factors appears to have increased the occurrence of cannibalism among lobsters (Oppenheim and Wahle, 2003). Increased cannibalism among age classes of walleye pollock (*Theragra chalcogramma*) has also been suggested to arise as a result of changes in atmospheric circulation over the Baltic Sea (Walther et al., 2002). Walther et al. (2002) noted that subsequent interactions between atmospheric circulation and ocean currents result in reduced transportation of young fish away from adults, increasing the occurrence of cannibalism.

Carey and Alexander (2003) have suggested that the impacts of climate change on amphibians had previously received relatively little attention due to the complexity involved in predicting future outcomes of global climate change. More recent studies have suggested links to increased pathogen transmission, altered trophic ecology and habitat conditions, and

overall population declines (Blaustein et al., 2010). However, due to the inherent complexity in climate change it is relatively difficult to predict how it will influence cannibalism.

One prediction is that climate change could lead to increased spread of pathogens and/or reduced immune function in amphibians leading to an increase in epidemics (Blaustein et al., 2010). Worthylake and Hovingh (1989) observed that populations of tiger salamanders suffered mass mortalities in four lake systems that had experienced dramatically reduced water levels as well as pathogenic bacterial blooms. Previous studies have indicated that such epidemics would innately reduce cannibalism by disproportionately killing off cannibals (Pfennig et al., 1991; Pfennig et al., 1998). Conversely, Bolker et al. (2008) suggest an interaction between cannibalism and infection, with one limiting the occurrence of the other and only extreme rates of infection of both larval and adult populations simultaneously would be capable of reducing cannibalistic traits.

This provides one example of the inherent complexity involved in trying to predict the future influence of climate change on cannibalism. Given that we do not fully understand the dynamics of the pathogen-cannibalism interaction it is difficult to predict future outcomes when the effects of climate change are factored in (Blaustein et al., 2010; Bolker et al., 2008; Collins and Storfer, 2003; Pfennig et al., 1991; Pfennig et al., 1998). However, while climate change is expected to have an overall negative impact on amphibian populations, the changes in the occurrence of cannibalism may or may not play an important role in such declines (Blaustein et al., 2010; Collins and Storfer, 2003).

In addition to the influence of climate, the chemical composition of wetlands could potential influence the expression of cannibalism. Ortiz-Santaliestra et al. (2012) observed that exposure to the common fertilizer ammonium nitrate removed the density-dependence of cannibalism among larval fire salamanders (*Salamandra salamandra*). By removing density-dependence effects, the authors' suggest the inherent benefits of cannibalism within natural

populations may be lost, such as that of reduced competition (Ortiz-Santaliestra et al., 2012). Although this is the only example of an environmental pollutant resulting in increased cannibalism I am currently aware of, it is possible that other pollutants and pesticides could potentially influence cannibalism in ways we are not currently aware of. It is also possible that in most instances, frequency of cannibalism will actually decrease with exposure to chemical pollutants and pesticides due to their toxicity resulting in increased mortality within affected populations. For example, Ortiz-Santaliestra et al. (2012) also identified that exposure to ammonium nitrate could dramatically reduce survivorship of Agile frog tadpoles (*Rana dalmatina*). However, the behavioural effects of pollutants and pesticides on larval amphibians should be further explored, including their potential influence on cannibalistic behaviour.

## **6.7 Conclusions**

Cannibalism may result strictly out of opportunity where vulnerable conspecifics may be consumed by larger conspecifics. It may arise out of necessity where starving individuals resort to cannibalism and it may result out of intense competition (Maret and Collins, 1997; Petranksa and Thomas, 1995; Reinhardt et al., 2015). Feeding aggregations provide opportunity as potential prey gather in proximity to putative cannibals (Wells, 2007). Additionally, forming aggregations around food should also prime individuals to feed. Feeding may escalate to agonistic behaviour as competition increases, and individuals may “displace conspecifics feeding on an attractive food source” (Waldman, 1985). Rather than simply pushing conspecifics away from food, individuals may escalate to biting at the tails, limbs, or gills of conspecifics (Waldman, 1985; Wildy et al., 2001). This behaviour may simply allow the individual to exploit the available food; however this may also escalate to partial or complete cannibalism. Benefits of this behaviour may subsequently allow certain

individuals to monopolize profitable food and feeding areas (Waldman, 1985). These individuals could then realize greater competitive success, and achieve growth and developmental benefits, and in turn provide size dependant advantages over conspecifics, and potentially further promote cannibalism (Church and Sherratt, 1996; Crump, 1983; Crump, 1990; Michimae and Wakahara, 2001; Wildy et al. 2001). As larval density increases the probability that aggression and agonistic behaviour among individuals should also increase due to proximity and opportunity. Expression of adaptive morphologies in responses to high population densities that may provide competitive advantages in obtaining food may also facilitate cannibalism (Collins and Cheek, 1983; Michimae and Wakahara, 2001; Walls et al., 1993). These behavioural and morphological adaptations should provide individuals a competitive advantage in a habitat that is inherently variable, and collaterally allow for cannibalistic behaviour.

From an evolutionary perspective, cannibalism may arise and persist because of its potential benefits to individuals under severe conditions (Fox, 1975a; Polis, 1981). Cannibalism and associated traits may therefore benefit larvae by increasing the population's dietary niche width through individual specialization and resulting in both direct and indirect reduction in competition, and thereby improving growth and development of survivors (Maret and Collins, 1997; Polis, 1981). Where cannibalism becomes too intense, selection pressure should result in species adaptations that reduce its frequency, such as synchronized breeding (Petranka and Thomas, 1995). Alternatively, spread of pathogens may innately limit cannibalism by infecting and killing cannibals (Pfennig et al., 1991; Pfennig et al., 1998). However, this spread of pathogens could have serious consequences to the entire population.

The results of this research suggest larvae become cannibalistic in response to competition. It appears that cannibalism is the result of escalating aggression in response to the crowded conditions that can arise within the temporary wetlands in which larvae develop.

Where wetlands may quickly evaporate and larval densities rapidly increase it may be necessary for individuals to adapt more aggressive competitive behaviours, which could lead to cannibalism. If climate change results in drier, warmer weather patterns this could result in pond conditions that increase the frequency of cannibalism and could have unpredictable consequences to global amphibian populations (Pfennig et al., 1991; Pfennig et al., 1998).

## 6.8 Future Directions

Although the results of this research provide advances in our understanding of cannibalistic behaviour there are still many unknowns. For example, while my research failed to show ideal growth and development in tadpoles fed conspecific tissues, I cannot be certain cannibalism is not still a high quality diet that is merely avoided by cautious individuals [Jefferson et al. 2014b (Chapter 2)]. To further explore whether such tissues can be efficiently digested by tadpoles, it may be useful to perform similar feeding experiments using heterogenerics such as species of *Pseudacris* (chorus frogs) that commonly co-exist with developing wood frog tadpoles. These species typically lack the unpalatable chemicals that tadpoles of many *Lithobates* species produce in their skin to deter predation from larger vertebrates such as fish, and salamanders (larval wood frogs lack such chemicals) (Szuroczki and Richardson, 2011). If wood frog tadpoles fed a diet of heterogeneric tissues exhibit growth and development that is greater than or similar to tadpoles fed a high quality diet (i.e. brine shrimp), then it stands to reason that the efficacy of conspecific tissues as a diet is hampered by behavioural inhibition in consuming such a diet and not necessarily the quality of the diet itself. Additional analyses of carbohydrate and lipid content of tadpoles should be conducted, along with calorimetric analyses to determine how conspecific and heterospecific tissues compare nutritionally relative to alternative experimental diets.

The nutritional stress larval amphibians experience when they are dependent upon low quality diets over time should also receive additional attention. Results of Experiment 2 in Jefferson et al. (2014b; Chapter 2) suggest that short term dependence upon a low quality diet is insufficient to produce a significant increase in whole body corticosterone. However, the longer term impacts to tadpole growth, development, and survivorship observed by McCallum and Trauth (2002) suggest that larvae could potentially exhibit increasing stress over time. McCallum and Trauth (2002) noted that survivorship to metamorphosis was much lower in tadpoles fed a diet that consisted solely of cornmeal, that these tadpoles exhibited lower mass, and had longer developmental periods. Audo et al. (1995) observed that nutritional stress at early larval stages could result in longer developmental periods to facilitate growth, however when faced with the same nutritional stress closer to metamorphosis development was not prolonged and tadpoles were forced to metamorphose at a smaller mass. The failure of my study to observe a stress response may therefore have been the result of an insufficient test period and/or testing only in the earlier stages of development where tadpoles could still recover from the negative effects of the poor quality diet. If dietary quality can initiate a stress response as suggested by Venesky et al. (2012) resulting in increased corticosterone levels, this could result in increased larval aggression in feeding and possibly lead to an increase in cannibalistic behaviour.

The hypothesis that cannibalistic traits can reduce competition within a population through individual specialization should be examined further (Maret and Collins, 1997). This would provide information on the effects of cannibalism on population and community ecology. Additionally, identifying whether such characteristics provide direct competitive advantages and/or serve to promote individual specialization within a population would improve our understanding of the evolution of cannibalism.



The effects of climate change on amphibians have previously received relatively little attention due to the complexity in climate modeling, and the inherent uncertainty in future environmental outcomes from such change (Carey and Alexander, 2003; Collins and Storer, 2003). However, the influence such change may have upon the expression of cannibalistic behaviour, and the associated characteristics should receive greater attention going forward. Long-term studies of natural populations and controlled lab based studies should be used to identify how temperature and precipitation influence the occurrence of cannibalistic morphology and behaviour. Impacts of altered rates of cannibalism should be examined to determine how this may influence populations and communities. Additionally, the potential interactions with cannibalism and pathogens should be assessed to determine what impact an increase in one or both may have upon populations.

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